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The journal was initially name as Aljouf University Medical Journal (AUMJ). As the university name changed into Jouf University, the journal's name was also modified into Jouf University Medical Journal (JUMJ) starting from the 3rd issue of the year 2018.

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Review Article

Bite force and implant therapy: A literature review

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Abstract

Background: Dental implants bite force (BF) is one of the most important parameters to influence masticatory efficiency and thus patient satisfaction. Therefore, it is essential to evaluate and assess BF particularly as it may play a significant role in disruption of the masticatory system.

Objective: The aim of this study was to thoroughly search of the literature for studies on factors influencing the BF of dental implants as well as studies that discuss methods of measurement.

Methods: A literature search was carried out in the following electronic databases, PubMed, Scopus, Embase, Google Scholar, Directory of Open Access Journals and Cochrane electronic databases. Keywords including maximum bite force, implant occlusion, dental implants bite force, jaw muscles, measurement devices were used to search these various databases.

Conclusion: The assessment of implant BF is a predictable method of evaluating the biomechanical properties of the masticatory complex as well as the efficiency of prosthodontic treatment.

Keywords: Implant, Bite force, Bite force gauge, Implant occlusion.

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Introduction

Successful dental implant therapy is the one which restores the function of the teeth as their original counterpart, which includes phonetics, mastication, aesthetics, and a variety of other functions⁽¹⁾. Under usual conditions, a unique freestanding tooth or implant is routinely exposed to masticatory forces that are usually compressive. While the majority of the treatment modalities based on implants have been historically concentrated on fixed prosthodontic tooth restoration, the myriad of advantages to the edentulous subjects from implant overdentures is astounding in terms of enhanced function, emotional balance, physical health and aesthetic component⁽²⁾. Appropriate appraisal and treatment planning of the completely edentulous subject have been shown to result in an enhanced quality of life for

patients and predictable outcomes resulting in clinical success.

The maximum bite force (BF) level in complete denture wearers has been restricted in amount because of the sensitivity or tenderness in the mucoperiosteum overlying the mandibular edentulous ridge which usually gets interceded between the dentures and bone⁽³⁾. Complete denture wearers seemed to be affected by more recurrent pain in the mandible compared to the maxilla⁽⁴⁾. Furthermore, a difference in the maximum BF level was noticed when comparing conventional dentures with implant-retained mandibular overdentures⁽⁵⁾.

Implant-retained dentures have several benefits when compared with traditional dentures. The inconstancy of mandibular dentures aggravates the acerbity of mandibular ridge atrophy. Physical

retention is restricted in subjects with pronounced resorption of the bone, as the aiding surface is extremely minimized, and stability of the mandibular denture is mainly contributed by the activity of the muscles. Anatomical constraints may limit the adoption of a fixed implant-retained prosthesis. In such cases, a removable implant-retained denture with a minimal number of implants may be considered⁽⁶⁻⁸⁾.

BF is considered as a decisive variable to explore the activity associated with dentition, occlusal factor, dentures and therapy using the implants, temporomandibular disorders, orthognathic surgery, and neuromuscular alterations⁽⁹⁻¹¹⁾. The muscular forces and the sum of functional teeth are decisive factors in masticatory functions. Estimating maximum BF is a bid to appraise the force resulting from the elevator muscles of the mandible. BF is a result of the activity of muscles related to maxilla and mandible, conclusively disseminated via the teeth on the object which is being chewed. The forces which result essentially during chewing activity influence the jawbones in varying dimensions, and they depend on the activity of musculature that cause an unambiguous action^(12, 13).

BF differs between people, and different sets of teeth that are being used for chewing. Therefore, this factor should be taken into consideration during dental surgery because of its influence on prosthodontic aspects like implant design, durability and adequacy of artificial teeth^(14, 15).

BF indicates the functional condition of the masticatory complex resulting from the activity of elevator muscles of the jaws. Estimation of particular BF level helps in setting up a proper treatment plan and also it assists in choosing the suitable type of prosthesis. Apart from this, BF may be considered as a significant factor in evaluating the disruption of the masticatory system⁽¹⁶⁾.

Analyzing BF is carried out directly with BF gauge transducer located amid a set of teeth. This straightforward approach, used for appraisal of the force, seems to be a conducive method for evaluating the

submaximal force⁽¹⁷⁾. An alternative approach is an assessment of BF indirectly by employing distinct physiologic variables that are well known to be practically applicable to force generation. Electromyographic function of the elevator muscles of the mandible can be culled up from the skin projection of the muscle belly. On these grounds, the resultant data provides a concept regarding BF. The observation of various researches revealed a linear association relating the potentials of electromyographic actions and BF which is measured directly; notably at submaximal grades⁽¹⁸⁻²¹⁾. Various elements impact the explicit BF evaluation and researchers noted varying values of maximal BF spectrum. The enormous difference in the values of BF relies on various circumstances pertinent to the anatomic and physiological attributes of the individuals. A far from the elements mentioned, the veracity and definiteness of BF values are influenced by the mechanical factors of BF recording system. In this work, the authors accentuated the significant factors that influence BF measurements, like age, sex, craniofacial morphology, periodontal apparatus, temporomandibular joint (TMJ) disorders, and dentition status. Ancillary to these components, mechanical elements composed of various recording devices, location of these devices in maxillary or mandibular jaws, measurement side with the aid of acrylic splints and wide opening of mouth were also emphasized by various studies related to implants and BF⁽²²⁻²⁵⁾.

The aim of this review was to highlight the important factors that influence the clinical value of BF and the various methods used for its measurement. Furthermore, it aims to explore factors relevant to BF measurement.

Methods

The following electronic databases, PubMed, Cochrane electronic databases, Scopus, Directory of Open Access Journals, Embase, and Google Scholar were consulted for the up-to-date literature search used for this review. MeSH terms used were a combination of the following: "maximum", "bite force",

“implant” “occlusion”, “dental”, “jaw muscles”, “measurement”, and “devices”.

Results & Discussion

Physiologic and morphologic variables affecting BF values

Age

Ageing process may lead to the depletion of orofacial musculature force. Certainly, the jaw closing force is increased with growth, and from 20 years till 50 years of age it is constant, with depletion afterwards. In young individuals with age range six to 18 years during the mixed and permanent dentition stages, it was found that the BF is positively correlated with age⁽²⁶⁾. Reports in the literature stated that BF decreases considerably with age, especially in females after 25 years, and in males after 45 years^(27,28). Among a Japanese population, maximum bites force, pressure magnitudes, and occlusal contact positions in young and elderly have been evaluated, and it was observed that these variables were much higher in the elder subjects. No diversity was revealed in combined occlusal forces and occlusal forces sharing among younger and elderly aged individuals due to wider teeth contact areas. Albeit the compelling interrelationship among age group and BF, the influence of age on BF is comparatively minimal⁽²⁹⁾.

Gender

Higher levels of maximum BF were noted in men compared to women^(10,30). The higher muscular ability of men is credited to the structural divergences. The masseter muscles in males have larger diameter and sectional area with different type of fibers when compared to females. Hormonal variability related to gender is reflected on the content of the muscle fibers. However, the interaction of sex and maximum BF will be manifested after 18 years of old^(10,30,31). It is well known that maximum BF surge all over the growth phase and development period without sex distinction. After the puberty, maximum BF surges at increased levels in men in comparison to women and this can be related to gender. Some researchers documented greater BF levels among men and attributed this outcome to a greater arch and tooth size of the individuals⁽³²⁾.

As the bigger arch and tooth size confer maximum periodontal ligament areas, it may contribute to a maximum BF. However, some researchers did not find compelling divergence in BF among females and males. This finding may be attributed to the minimal number of study participants, and to the analysis of functional forces arising at sleeping period. Although some researchers have found that the gender has no effect on the BFa; the diversity in the values of BF between females and male has been spotted by most of the studies^(19,27,33).

Cranio-facial anatomy

Variation is noted in maximum BF according to the skeletal measurements of the craniofacial pattern of the individuals, which includes the ratio of posterior and anterior facial height, mandibular angle and the inclination of the mandible^(34,35). BF reflects the geometry of the mandibular lever system. In cases where the ramus of mandibular is more horizontal and gonial angle is obtuse, elevator muscles of the mandible may not contribute mechanical benefits. An inverse relation between BF value and inclination of the mandible was observed previously⁽³⁶⁾. This was in accordance with other studies which found that the long-facial type of the craniofacial morphology was correlated with limited BF values. The following variables were found to affect BF positively: muscular thickness, thickness of masseter and temporalis, and morphology of the face. Thicker masseter muscles were observed in people with a short face when compared to normal or long-faced people, whereas short-faced subjects may demonstrate substantial BF's⁽³⁷⁾.

Periodontal status

Loading forces during the masticatory phase which were generated from muscles of mastication were governed by the mechanoreceptors located in the periodontal ligament⁽³⁸⁾. So, decreased support from periodontal tissue can reduce the threshold level of the mechanoreceptor's activity⁽³⁹⁾. This state may lead to alterations in the biting. It has been mentioned that subjects with loss of attachment may show altered sensory function which may result in decreased

regulation of biting force⁽³⁹⁾. It has been stated that biting capacities of persons having normal periodontal conditions were notably increased in comparison to individuals having chronic periodontal diseases. Whereas, a positive correlation has been observed between biting ability and periodontal status, a minimal influence of periodontal status on the capacity of biting has been observed. It has been reported that compromised periodontal status will not restrict the BF with maximal fortitude in subjects with natural teeth⁽³⁸⁾. Furthermore, it has been noted that the reduced number of periodontal neural receptors may be sufficient for appropriate feedback action restricting BFs and masticatory force. The disparity among these studies is perhaps attributed to variations in measurement areas and appliances used for recording the BF's^(40,41).

Disorders of the temporo-mandibular joint

TMJ disorders (TMJDs) is comprised of multiple clinical manifestations affecting orofacial system of masticatory muscles, and TMJ with subsequent debilitation of these structures⁽⁴²⁾. BF influences the efficiency of the stomatognathic system; therefore, evaluation of BF might be of a beneficial complementary mode of perceiving masticatory activity among subjects having orofacial disorders. So, various practitioners concentrated on BF to actuate if there is any effect of BF among patients with temporomandibular disorders. A significantly lower BF in patients with TMJDs was observed when compared to healthy subjects⁽³⁶⁾. The authors considered that pain in masticatory muscles along with inflammation of temporomandibular joint can lead to limitation of maximum BF. It has been stated that TMJDs was the common etiologic factor for limiting BF⁽³⁶⁾. In confirmation with these observations, a significant correlation between reduced BF and tenderness of musculature, and pain in TMJ was revealed⁽³⁶⁾. However, other researchers found no significant discrepancies in maximal BF values between healthy subjects and patients with TMJDs. These alterations in observations may arise from

the sourness of the TMJDs in subjects or contrasting recording techniques⁽⁴³⁾.

Bruxism is a significant causative factor leading to TMJDs, usually represented by clenching and grinding the teeth. Few authors compared the BF strength in bruxists utilizing a Gnathodynamometer and observed that bite strength in some bruxists was significantly higher when compared to non-bruxists. Other authors have estimated BF levels using a load transducer in both bruxists and non-bruxists and noted that both categories had no alterations in maximal BF values⁽⁴³⁾. However, despite the fact that the height and properties of transducers in those studies were the same but the degree of bruxism and the diagnostic procedures might not be the same.

Dentition and restorations

Dentition with associated restorations, fixed and removable dentures, and number and position of existing teeth are important determining factors of BF. A positive correlation between the number and position of the dentition at both maximal and submaximal BF has been observed⁽¹⁹⁾. The number of existing dentition and their contact seem to significantly influence the maximum BF. The larger BF in the posterior aspect of the jaw may be reliant on heightened contact area and the number of posterior teeth loaded while biting. For instance, occlusal contact areas double in cases where maximum BF levels increased from 30% to 100%⁽⁴⁴⁾. Some researchers evaluated the values of occlusal BFs among the population having restorations in incisors and molars and compared with those of non-restored counterparts. The subjects with restorations had significantly minimal BF in the incisor region when compared to molars. As per the data observed in that research, the authors claimed that it may be conceivably as a result of the adaptations to the restorations⁽⁴⁵⁾. Some investigators compared maximum BF levels in persons with fixed partial dentures (FPD), removable partial dentures (RPD) and complete dentures (CD) and those having a completely natural set of teeth. The subjects having natural teeth revealed maximum BF levels, the biting forces

observed were 80% for FPD, 35% for RPD and 11% for CD wearers⁽⁴⁶⁾. When comparing BF in subjects having, partial dentures, and those with natural dentition, it was observed that the maximum BF was exhibited by the subjects that have natural dentition. A downfall in the BF was observed in the location of advanced alveolar process resorption and a significant correlation between the height of the alveolar process and the BF⁽²³⁾. Some researchers have correlated BF levels among subjects having dentures supported by implant, remaining roots, CDs and those having existing natural teeth. Individuals having dental implant-supported overdenture revealed forces much greater when compared with the subjects having CDs and root-retained dentures. Nonetheless, maximum BF's employed by the subjects with implants were minimal when compared with individuals with natural dentition⁽⁴⁷⁾.

Measurement of the biting force

Recording devices and techniques

Research interest in intraoral BF had been recognized for a very long time and discussed thoroughly in literature. In relevant studies, a variety of techniques as well as equipments to estimate BF were reported. These equipments range from a simple spring to a wide variety of complex electronic appliances. Borelli in 1681 performed the first study defining the intraoral forces carried out by those who fabricated a Gnathodynamometer⁽³⁸⁾. The first scientific examination of BF was carried out in 1893 by Black. He got his results by fabricating the latest variety of Gnathodynamometer. Various scientists later extended investigation regarding this topic and fabricated the lever-spring, manometer-spring and lever, and micrometered appliances. In today's practice, sensitive electronic devices are commonly employed which are reliable and accurate for the analysis of routine load. The action of all the latest equipment is dependent on the strain-gauges electrical resistance⁽⁴¹⁾.

Gnathodynamometers are routinely adopted for measuring BF for an extended period and some researchers apply strain gauges mounted dynamometer for BF documentation. The digital dynamometer is the latest version which uses electronic

technology and is composed of the digital body and a bite fork. Its increased precision load cell and an electronic circuit to demonstrate the force, is known to provide accurate values⁽⁴⁸⁾. Recently, piezoelectric film that is very sensitive to deformation was adopted as a force sensing documenting system. The piezoelectric film deformation induces an electrical signal that differs according to the force induced to the film. An amplifier is intended to intensify the piezoelectrical signal on the grounds that the created signal is a very minimal electrical current. This device was used in a study where the current was conveyed to a digital recorder in which the level could be recorded directly or with the help of graphic recorder. The detector was commented to the amplifier and then to a circuit where the output electrical signal was conveyed to a PC framework⁽⁴⁹⁾. A more recent small BF recorder was later introduced; it was a silicon beam semiconductor that apportioned in the form of a tactile unit. A load over the sensor induces corresponding amendments in the dual resistors and advances to electric modifications in the circuit. Its standardization test has revealed acceptable reliability with BF's in the range of 10 to 1000 N. A conductive polymer pressure-sensing resistor with a 12 mm diameter and of 0.25mm thick was used; it was composed of two conducting interdigitated electrodes facing another sheet that is coated with a semi conductive poly-etherimide ink⁽²⁹⁾.

Strain-gauge BF transducer is the utmost universally adapted recording appliance and it is available in different heights and widths. BF was measured using a strain-gauge transducer 4 mm in height and 5x7 mm in width, its standardization was carried out at room temperature between the force of 0 and 350 N, with an error of $\pm 2\%$. The linear deviation was $\pm 7.3\%$ and $\pm 9\%$, with a load of 300 N and 350 N, respectively. The maximum variation of BF was noted to be stratified between forces of 446 N and 1221 N⁽⁴⁷⁾.

A dental pre-scale system consisting of a horse-shoe structured bite foil of a pressure-sensitive film (PSF) and a computerized scanning system was used

to analyze the load. At the point when forces were induced over occlusal contact; a chemical reaction occurred which produced a graded color. The uncovered pressure-sensitive foils were scrutinized through the occlusal scanner, which interprets the area and color intensity of the red dots to determine occlusal contact area and pressure. Two different types of pressure-sensitive sheets exist: Type R and W. The R type is 97 μm thick whereas the W type is 800 μm thick. Both sheets are then classified into either 30 H or 50 H. The combined occlusal load evaluated with PSF and unilateral strain-gauge transducer has been compared. A maximum BF was registered with a strain-gauge transducer positioned over mandibular first molar teeth in a 6 to 7 mm bite opening. A 0.097 mm thick horse-shoe shaped pressure-sensitive foil with maximum BF registered in the intercuspal position area. The chance of estimating BF from each tooth in documenting with a little brawl to the occlusion is granted by the thin pressure-sensitive foil. The traditional type of this recording system adopts the analysis of the combined jaw-closing at particular focuses over the dentition; the occlusal position is altered by the inevitable jaw partition and limits the occlusal support lead by the device. The diversification between total most extreme UT force and most extreme PSF force have been elucidated by a technical constraint in a computerized scanning device of a dental prescale unit. Even though the changes in the outright benefits of closing force, the combined maximum PSF forces and UT forces have been noted to be associated; PSF forces at mandibular first molar, the mean PSF combined forces, and UT forces were documented as 148 N, 1109 N, 553 N, respectively. A bite-force analyzing system like a dental prescale system adopting thin PSF is known to be superior to routine analyzing systems that function by using a strain-gauge transducer. This result may be substantiated by two facts. First, BF can be recorded near to intercuspal position, which contributes a good chance to analyze BF in normal circumstances. The second one is the load sharing along the

dentition can be analyzed in the meantime⁽⁵⁰⁾.

BF applying a strain-gauge BF and a conductive polymer pressure-sensing resistor (force-sensing resistor) were compared. BF recordings retrieved from both the systems have revealed significant differences in BF levels. The authenticity of the sensor to register reproducible force levels amid two different loading series was noted by about 93%. The observations of unaltered loading tests have revealed that the novel BF sensor proved capable to document intraoral forces satisfactorily with precision and veracity. Researchers expressed a few challenges related to the BF sensor⁽²⁹⁾. The significant is the load rate-dependent characteristics of sensor and its nonlinearity, which can be interpreted slightly by an assertive extent of the nonlinearity of the sensitive resistor and its surface material devastation⁽²⁹⁾.

Location of the recording device

BF differs in various locations of the oral cavity; the greatest BF is recorded when the transducer is placed posteriorly⁽⁵¹⁾. This may be attributed to the mechanical lever system of the jaw. Increased BF may be well tolerated in the posterior dentition due to their increased surface area and periodontal ligament support. In cases where BF transducer is placed in the most posterior position, the anterior fibers of the temporalis muscle will turn out to be more dynamic making a more maximum endeavor to the effort⁽⁵²⁾.

Measurements at different sites

Recording at the unilateral or bilateral application is another factor that determines the value of BF. Majority of studies revealed that BF is greater during bilateral clenching compared to unilateral clenching⁽⁵³⁾. When traditional force transducers were applied in normal persons, using unilateral and bilateral clenching, bilateral total BF was noted to be 40% more than unilateral clenching. Unilateral and bilateral BF analysis was compared with various transducers, employing a pressure-sensitive foil of a 0.1 mm thickness for bilateral clenching and a 6 to 7 mm thick conventional force transducer for unilateral clenching. 100%

acceleration in BF and a 50% increase in masseteric action during bilateral clenching in comparison to unilateral clenching were recorded. BF and actions of jaw muscles were analyzed using strain-gauge transducer and a 30% larger BF was observed when measured bilaterally. When compared to unilateral measurement 30% higher actions of right and left masseter and the anterior part of the temporal muscles were documented. No variations in the action of masseter muscles in the unilateral clenching experiments were observed, whereas a difference in the actions of left and right temporal musculatures during unilateral clenching and the loaded side revealed increased activity of muscles⁽¹⁹⁾.

The jaw musculatures shall induce a unilateral BF which is equivalent to the resultant force generated during clenching bilateral. In comparison to half of the force when recorded bilaterally the force on each side is more if analyzed unilaterally. Actions of the muscles attached to the mandible and BF retrieved in unilateral clenching may be a product of the periodontium and the inhibition of TMJ receptors. In order to minimize the trauma related to the dentition, receptors inhibition of periodontium limits the extremely strong BFs and increased muscular activities⁽⁵²⁾.

Role of Acrylic splints

Acrylic appliances were adopted to safeguard the cusps of the teeth, to avert dental fracture during maximum clenching. BF levels were compared in subjects with and without splinting and it was revealed that BF levels are increased by using acrylic splints⁽⁵⁴⁾.

Opening of Jaw

Alterations in the orofacial structures were observed from the increase in the vertical dimensions altering the length of the elevator musculatures of the jaws and the position of the head of the mandible. A decreased value of the electromyographic activity of masseters with jaw opening was noted when BF values were maintained constant. A maximum BF magnitude was documented between 15 to 20 mm of anterior vertical jaw opening when constant levels of

masseter muscle activity maintained. The maximum incising force has been found at peak levels at the incisal opening of 17 mm. A decrease or increase in jaw separation was observed from this optimum opening and a decrease in the strength of the maximum incising was also noted. The results of this research revealed that the population means for induction of the intense BF was in the range of 14 and 20 mm of jaw separation⁽⁵⁵⁾.

Implants BF

The evolution of root-shaped dental implants and osseointegration theory allowed for replacement when teeth are missing with increased analogy. This also permitted unravelling of the problems with the adoption of removable prosthesis such as limited BF. Loss of teeth and the subsequent replacement will decrease BF when compared to natural teeth, and it is also correlated with other complications like conditional loss of the bone. Dental implants are known to enhance BF as a component of masticatory ability in subjects having severe resorption of the alveolar ridge. Subjects with mandibular ridge resorption and with implant-supported prostheses exhibit similar masticatory efficiency as those patients with the normal alveolar ridge. It has been widely stated that BF is increased greatly with dental implants⁽⁵⁶⁾. Al-Omiri et al assessed maximum occlusal BF in subjects with fixed bridges supported by implants in comparison with the opposite side having teeth and also to evaluate how the maximum occlusal BF is influenced by age, sex and body mass index (BMI)⁽⁵⁶⁾. The authors noticed a higher average maximum occlusal BF at the dentulous side, in males with higher BMI. The maximum occlusal BF levels were significantly higher at the dentate side in comparison to the implant-supported prosthesis side. BMI was not significantly correlated with maximum occlusal BF. Kayumi and his co-workers used finite element analysis to investigate the impact of occlusal forces employed during occlusal modification on the distribution of the forces on dentition, dental implants, and TMJs during intercuspal clenching, whenever there are missing posterior teeth

bilaterally⁽⁵⁷⁾. It was observed that the occlusal forces were concentrated on the most posterior implants while the load was larger, and the percentage of bearing force at TMJ was small. Rismanchian and his colleagues measured the maximum BF and evaluated patients' satisfaction from their conventional CDs and mandibular implant-supported overdentures opposed by CDs⁽⁵⁵⁾. The authors evaluated BF by using electronic BF measuring equipment having strain gauges. In the region of the first molar, three measurements were made on each side and the mean values were noted. Questionnaires were used for recording the patients' satisfaction. The authors observed a significantly higher maximum BF in the patients with mandibular implant-supported overdentures when compared to patients having conventional CDs. From this observation, it can be noted that dental implants play a significant role in the amelioration of BF and chewing ability leading to the satisfaction of the patients⁽⁵⁶⁾.

Conclusion

Assessment of BF has been a predictable method of evaluating prosthodontics treatment and the masticatory complex biomechanical properties. It is advisable to contemplate other compelling aspects when scrutinizing BF measurement. Implant-supported prostheses generate higher levels of BF and increased masticatory efficiency when compared with conventional dentures. Oppositely, less BF is observed with conventional dentures in comparison to overdentures. However, overdentures induce less BF than natural dentition. Higher rate of stability, BF, and masticatory efficiency may be experienced with the implant-supported denture in lieu of conventional denture.

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Conflict of Interests

The author declared no conflict of interests.

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Original Article

Antifungal Susceptibility Pattern of Clinical Isolates of *Candida* species at Khartoum State Hospitals, Sudan

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Abstract

Background: Antifungal resistance has been evolving lately as a burgeoning healthcare problem among *Candida* species.

Objective: This study was aimed to determine the susceptibility patterns of *Candida* species to four antifungal agents (Fluconazole, Itraconazole, Nystatin and Amphotericin B) using a disk diffusion method.

Materials and Methods: A total of 100 clinical isolates of *Candida spp.* were isolated and studied. They belonged to clinical samples of different types collected from hospitals and laboratories during the period from December 2018 to November 2019. The isolates were identified morphologically using basic cultural techniques, growth characteristics, and their growth inhibition susceptibility to Fluconazole, Itraconazole, Nystatin and Amphotericin B antifungal drugs using disk diffusion method.

Results: Gender-wise, most of the *Candida* isolates belonged to females compared to males (72 vs. 28%). Sample type-wise, the majority of *Candida* species (71%) were obtained from urine samples. *Candida albicans* was the most frequently isolated species (63%). A high resistance to Fluconazole (50%) 48% and Itraconazole (56%) 43% by *Candida* species was observed. *Candida* isolates from females showed the highest resistance rates and were mostly isolated from urine specimens. 96 (96%) of the isolates were Amphotericin B sensitive, while 2% of the isolates were susceptible; dose-dependent. For Nystatin, 56% of the isolates were sensitive and 42% were susceptible; dose-dependent. Statistically, it was found that the difference in susceptibility pattern between *C. albicans* and Non-*albicans Candida* species was insignificant among all tested antifungals except Itraconazole.

Conclusion: While our isolates were mostly sensitive to polyene antifungal agents, ~50% of them were resistant to azole antifungal agents particularly among urine samples from females. The high resistance rate to the commonly used azole antifungal agents implicates continued antifungal-susceptibility surveillance needs to be conducted to monitor the antifungal susceptibility trends of *Candida* species and other opportunistic fungal pathogens.

Keywords: Disc diffusion, *Candida* species, Clinical specimens, Antifungal agents, Susceptibility test, Khartoum State.

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Introduction

In the last decades, the occurrence of fungal infections has increased dramatically due to rise in the number of immunocompromised patients, higher numbers of people with AIDS, an

increasingly aged population and the more widespread use of indwelling medical devices⁽¹⁾. *Candida* species are the most common cause of fungal infections worldwide⁽²⁾. They are

endogenous opportunists which cause secondary infections in individuals with underlying immunocompromised conditions⁽³⁾. The infection may be superficial, local mucosal and sometimes systemic⁽⁴⁾. *Candida* species is the important cause of substantial morbidity and mortality in hospitalized patients. It is fast becoming a very important pathogen among critically ill patients⁽⁵⁾.

The availability of antifungal agents for the treatment of fungal infections is significantly lower, when compared with antibacterial agents. This is mainly due to the fact that fungi are eukaryotic, and thus identifying drug targets to selectively kill fungal pathogens without toxicity to the host is problematic⁽⁶⁾. For many years, the standard treatment for candidiasis has been amphotericin B. Recently, azoles are being used as initial therapy for *Candida* infections due to having different spectrum of activities, with low toxicity and high efficiency⁽⁷⁾. The intrinsic and emerging resistance to azoles actually represents a major challenge for empirical therapeutic and prophylactic strategies⁽⁸⁾. The antifungal susceptibility testing may specify clinical response, predict treatment failure and develop local antibiograms aiding in empirical selection of antifungal agents⁽⁹⁾. Therefore, the use of in vitro laboratory tests may aid the doctor in choosing an appropriate therapy⁽¹⁰⁾. Also, a rapid reliable susceptibility testing method would enable the clinician to prescribe the most suitable antifungal agent that avoids more toxic or expensive therapy⁽¹¹⁾.

To our knowledge there is a few data on the sensitivity pattern of candidiasis in Sudan. Thus, this study was aimed to detect the in vitro susceptibility pattern of *Candida* species to commonly used antifungal agents in Sudan using a disk diffusion assay which is a standard simple, rapid and reliable test.

Materials and Methods

Design and setting

This cross-sectional study encompassed different Khartoum State hospitals and laboratories (Fedail, Military Medical, and Soba Teaching Hospitals, and, Altra and Alborj Laboratories), during the

period from December 2018 to November 2019. A total of 100 clinical isolates of *Candida* species from different types of clinical samples (urine, high vaginal swab, sputum, ear swabs, catheter tip and blood) were considered. Data in the present study that included patient's gender, and age, and, type of clinical specimens were obtained from hospitals records. The bioethical approval was taken from Al-Neelain University Ethical committee (Approval number AU/FMLS/7/1).

Sample processing

Colonies from the positive cultured clinical isolates of *Candida* species were subcultured on Sabouraud Dextrose Agar (SDA) and incubated at 37 °C, for 24 - 48 hours to ensure purity and optimal growth, and then they were re-identified by morphology and microscopy. The morphology of *Candida* species colonies on SDA is white to cream, round, curved, soft and smooth to wrinkle with characteristic yeast odor. Air dried fixed smears were prepared and stained using Gram's stain. The stained slides were examined under light microscope using 100X oil immersion objective to observe the gram positive budding yeast cells.

Germ tube test

Using a sterile inoculating loop, a colony of yeasts was transferred into the human serum in the labeled test tubes. The colony was emulsified in the serum and incubated at 37 °C for 2 hours (if germ tubes are not seen after 2 hrs, the test was considered negative). If germ tubes (germinating hyphae, short and aseptate) were seen on the slide under the microscope, test result was recorded as positive which confirmed the presence of *C. albicans*. Absence of germ tube was noted as negative result showing that it is a non-*albicans* species of *Candida*⁽¹²⁾. We considered at least five germ tubes be observed in the entire wet mount preparation to label isolate as germ tube producer. Negative results were confirmed by examining minimum 10 high power fields as previously reported⁽¹³⁾.

Disk diffusion susceptibility test:

Antifungal susceptibility testing was performed as per NCCLS M44-A2 guidelines⁽¹⁴⁾ for disk diffusion method without using control stains. The inoculum was prepared from the 24-hours old culture on SDA and the turbidity was adjusted to 0.5 McFarland standards with approximately $1 - 5 \times 10^6$ CFU/mL. Within 15 minute after the inoculum suspension was prepared, a sterile swab was used for inoculating the dried surface of a sterile GMB-Mueller-Hinton agar plate (Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/mL methylene blue). After 3 - 5 minutes, the antifungal disks for Fluconazole (25 µg), Itraconazole (8 µg), Nystatin (100 U), and Amphotericin B (10 µg)) were applied by sterile forceps (Rev.7/03.12.2012; Liofilchem® S.r.l., Roseto degli Abruzzi, Italy). Each disk was pressed down to

ensure complete contact with the agar surface. The plates were aerobically incubated at 37 °C for 24 hours. The diameter of the growth inhibition zone around each antifungal disk was manually measured by a ruler and was recorded. The results were interpreted as Sensitive, susceptible; dose-dependent, or Resistant (Table 1) as previously reported using the manufacturer's antifungal drugs⁽¹⁵⁾.

Statistical analysis

The collected data with the laboratory results were analyzed by the statistical package of social science (SPSS) soft program version 20, with a reference significant p value ≤ 0.05 . Frequencies and percent obtained in frequency tables, Chi-square test for goodness of fit was used to test these frequencies. The relationship between variables was tested using cross tables and Chi-square (Fisher Exact) test for independence.

Table 1: Interpretation of antifungal disk diffusion susceptibility test results.

Antifungal agents	Disk potency	Growth Inhibition Zone Diameter, mm		
		Sensitive	Susceptible; dose-dependent	Resistant
Fluconazole	25 µg	≥ 19	15 - 18	≤ 14
Itraconazole	8 µg	≥ 16	10 - 15	< 9
Nystatin	100 IU	≥ 25	17 - 24	< 16
Amphotericin B	10 µg	> 15	10 - 14	< 9

Results

A number of 100 *Candida* clinical isolates collected from different Khartoum State hospitals and laboratories (Fedail Hospital, Military Medical Hospital, Soba Teaching Hospital, Altra Lab and Alborj laboratory) were

subcultured for identified and antifungal susceptibility testing. Representative SDA cultures of isolates that show the different morphologies (Figure 1), microscopic appearance of germ tube positive isolate (Figure 1 insert), and growth inhibition assay are presented in Figure 2.

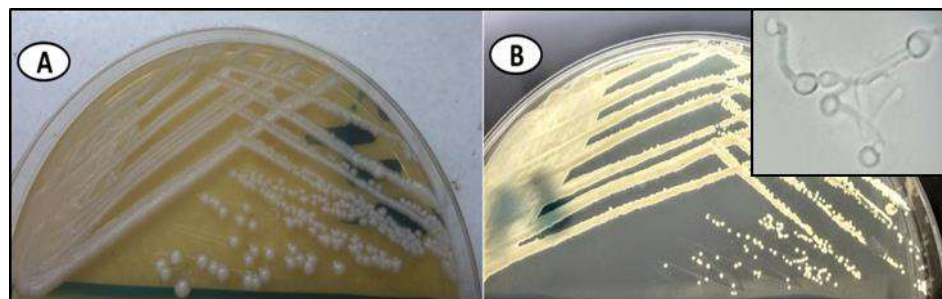


Figure 1: Example cultures showing the different colonial morphology of *Candida* species on Sabouraud Dextrose Agar. A. Large, convex, opaque, round and moist *Candida* colonies. B. small, flat, opaque, round and moist *Candida* colonies. Figure insert: Example microscopic image of *Candida albicans* with positive germ tube test.

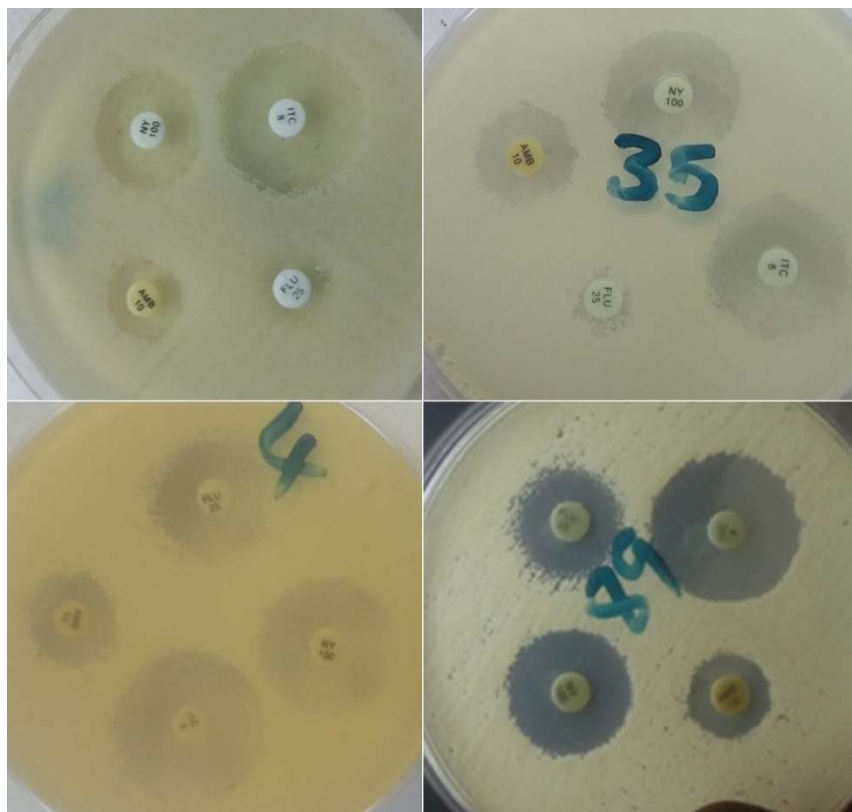


Figure 2: Example antifungal susceptibility pattern of *Candida* species to Amphotericin B (AMB), Fluconazole (FLU), Itraconazole (ITC) and Nystatin (NY).

Distribution of clinical isolates of Candida species:

The isolates were collected from both male and female patients with age range of 3 month - 96 years (mean age was 43.7 years). The majority of samples were collected from patients aging 21 - 40 years (Table 2), and among females more than males (Figure 3).

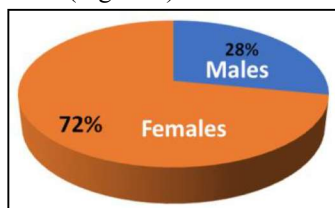


Figure 3: Frequency of *Candida* species positive clinical samples stratified to gender of patients. Data shown are %.

The 100 isolates were obtained from different types of clinical samples; 71 (71%) urine, 16 (16%) high vaginal swabs, 7 (7%) sputum, 2 (2%) ear swabs,

2 (2%) catheter tips and 2 (2%) blood (Table 3).

Table 2: Frequency of *Candida* species positive clinical samples stratified to age of patients. Data shown are frequencies; n (%).

Age group, Years	n (%)
0 – 20	5 (5)
21 – 40	37 (37)
41 – 60	26 (26)
61 – 80	30 (30)
81 – 100	2 (2)
Total	100 (100)

Table 3: Frequency of *Candida* species positive clinical samples stratified to type of the sample. Data shown are frequencies; n (%).

Specimens	<i>Candida</i> Positive Samples; n (%)
Urine	71 (71)
High vaginal swabs	16 (16)
Sputum	7 (7)
Others	6 (6)
Total	100 (100)

According to Germ Tube Test, out of 100 isolates, 63 (63%) were *C. albicans* and 37 (37%) were non-*albicans Candida* species (Figure 5).

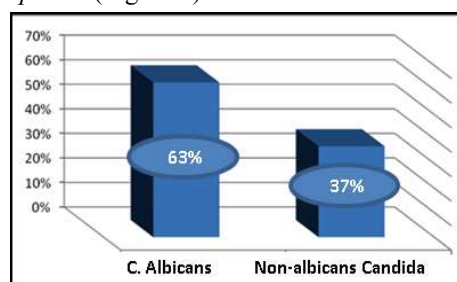


Figure 5: Frequency of the type *Candida* species according to Germ Tube Test result; *C. albicans* vs. non-*albicans Candida*. Data shown are %.

Susceptibility to azole antifungal agents (Fluconazole and Itraconazole)

Table 4: Susceptibility patterns of *Candida* species isolates to azole antifungal drugs; Fluconazole (FL) and Itraconazole (IT). Data shown are frequencies; n (%) and p value. S = Susceptible, SDD = Susceptible; Dose-Dependent, and R = Resistant.

Azole Group		C. Species		Total	p
		<i>C. albicans</i>	Non- <i>albicans Candida</i>		
FL	S	28 (44)	20 (54.1)	48 (48)	0.086
	SDD	0 (0.0)	2 (5.4)	2 (2)	
	R	35 (56)	15 (40.5)	50 (50)	
Total		63 (63)	37 (37)	100 (100)	
IT	S	20 (31.7)	23 (62.2)	43 (43)	0.004
	SDD	0 (0.0)	1 (2.7)	1 (1)	
	R	43 (68.3)	13 (35.1)	56 (56)	
Total		63 (63%)	37 (73)	100 (100)	

Table 5: Susceptibility patterns of azole; Fluconazole (FL) and Itraconazole (IT) antifungal drugs stratified to gender and type of clinical specimens. Data shown are frequency and p value. HVS = High vaginal swabs.

Antifungal		Gender		Total	p	Sample Type				Total	P
		Male	Female			Urine	Sputum	HVS	Other		
FL	S	15	33	48	0.56	35	3	7	3	48	0.97
	R	13	37	50		34	4	9	3	50	
	SDD	0	2	2		2	0	0	0	2	
Total		28	72	100		71	7	16	6	100	
IT	S	10	33	43	0.51	34	2	5	2	43	0.80
	R	18	38	56		36	5	11	4	56	
	SDD	0	1	1		1	0	0	0	1	
Total		28	72	100		71	7	16	6	100	

Susceptibility to polyene antifungal agents (Nystatin and Amphotericin B)

As shown in Table 6, 56 (56%) and 42 (42%) of the total tested isolates were sensitive and susceptible; dose-dependent

Table 4 shows that, among the 100 examined isolates, 48 (48%) were sensitive to Fluconazole, 2 (2%) susceptible; dose-dependent and 50 (50%) were resistant. The response against Itraconazole exhibited 43 (43%) sensitive, 1 (1%) susceptible; dose-dependent and 56 (56%) resistant isolates. The difference in Fluconazole susceptibility pattern between *C. albicans* and Non-*albicans Candida* species was insignificant ($p = 0.086$), while it was highly significant for Itraconazole ($p = 0.004$).

Most of Fluconazole and Itraconazole resistant species were isolated from female patients and among urine samples as compared to males and other types of clinical specimens (Table 5).

to Nystatin, respectively, whereas only 2 (2%) of isolates were resistant. The results of susceptibilities to amphotericin B indicated that most of the tested isolates 96 (96%) were sensitive and the rest of

them [4 (4%)] were susceptible; dose-dependent. The difference in susceptibility pattern between *C. albicans* and non-*albicans Candida* species was

insignificant against Nystatin and Amphotericin B ($p = 0.290$ and $p = 0.108$, respectively).

Table 6: Response of polyene antifungal drugs; Nystatin (NY) and Amphotericin (AMB) against *Candida* species. Data shown are frequencies; n (%) and p value.

Antifungal		Candida Species		Total	P
		<i>C. albicans</i>	Non- <i>albicans Candida</i>		
NY	S	39 (61.9)	17 (46)	56 (56)	0.290
	SDD	23 (36.5)	19 (51.3)	42 (42)	
	R	1 (1.6)	1 (2.7)	2 (2%)	
Total		63 (63)	37 (37)	100 (100)	0.108
AMB	S	62 (98.4)	34 (91.9)	96 (96)	
	SDD	1 (1.6)	3 (8.1)	4 (4)	
	R	0 (0.0)	0 (0.0)	0 (0.0)	
Total		63 (63)	37 (73)	100 (100)	

Discussion

Antifungal susceptibility testing of *Candida* is becoming recognized as a useful tool to optimizing the treatment of *Candida* infections to avoid the continuously emerging resistant strains⁽¹⁶⁾. Also preclinical and clinical studies found an association between the timely initiation of appropriate antifungal therapy and infection outcome which showed the importance of performing antifungal susceptibility testing in clinical laboratories⁽¹⁷⁾.

In the present study, the candiduria was found to be more common among females, it may be due to colonization of vulvo-vestibular area with *Candida* species, so they are at risk of developing ascending infection⁽¹⁸⁾. The highest number of *Candida* isolates were from urine samples (71%) and high vaginal swabs (16%), which is similar to Tasneem et al (2017)⁽⁷⁾ where they found urine and vaginal swabs had predominant *Candida* species. Similarly, the study by Aslam (2016)⁽¹²⁾ also showed that *Candida* was most abundantly isolated from urine (35.5%), followed by high vaginal swabs (29%).

In our study, *C. albicans* (63%) was the leading pathogen as compared to non-*albicans Candida* species (37%). Several other investigators also reported similar results such as the study carried in Ethiopia by Moges et al (2015)⁽¹⁹⁾ which showed that *C. albicans* was the most

frequently isolated species accounting for 68.4% of the total yeast isolates. Also, Elfeky et al (2016)⁽²⁰⁾ and Gandhi et al (2015)⁽²¹⁾ found that *C. albicans* was the predominant isolated species; comprising 60.3% and 66.39%, respectively. Many studies⁽²²⁻²⁶⁾ also reported *C. albicans* as the most frequent species. Nonetheless, a study done by Deorukhkar et al (2014)⁽²⁷⁾ showed a higher frequency of non-*albicans Candidiasis* (63.3%) in comparison to *C. albicans* (36.7%).

Our study found higher resistance rate against azole agents; 50% towards fluconazole and 56% to Itraconazole. Also a study done by Zaidi et al (2018)⁽²⁸⁾ showed significant resistance against fluconazole (56.5%) and Itraconazole (64.5%). The study done by Khara et al (2017)⁽²⁾ showed higher resistance rate against both fluconazole (63%) and Itraconazole (62%). Additionally, the study by Khan et al (2018)⁽⁹⁾ found that *Candida* species were exceedingly resistant to Fluconazole (62%). The resistant rate for Fluconazole in the present study was higher than studies done by Giri et al (2013)⁽²⁹⁾ and Dewan et al (2015)⁽³⁰⁾ where Fluconazole resistance rate was 30.8% and 20%, respectively.

The development of azole antifungals enhanced the treatment options for fungal infections, and their reduced host toxicity has led to their widespread use. Consequently, such extensive use made it unsurprising to see a high resistance rate

against them, particularly Fluconazole⁽¹⁾. Also, this study found that the resistance to azole was most common among females and the resistance species were mostly isolated from urine specimens. This may be due to the fact that urine samples were predominant compared to others or because Fluconazole was the first choice antifungal drug for candiduria. Therefore, the extensive and inappropriate use leads to appearance of more resistance.

Although the present study showed low resistance rate against Nystatin (2%), it has a high rate of susceptible; dose-dependent (42%). Similar study⁽²⁷⁾ showed that 14% rate of resistant and 80% dose-dependent susceptibility to Nystatin. The study done by Mahmoudabadi et al (2013)⁽¹⁵⁾ reported that 54.8% and 44.1% of the tested isolates were susceptible; dose-dependent and sensitive to Nystatin, respectively.

This study showed that the most effective antifungal agent used was amphotericin B, where 96% of the isolates were sensitive and 2% susceptible; dose-dependent. The higher Amphotericin B sensitivity was found in both *C. albicans* and non-*albicans* *Candida* species. Also, two other studies^(29,21) showed high sensitivity rate (100%) and (98.3%) against Amphotericin B, respectively. Similarly, Elfeky et al (2016)⁽²⁰⁾ reported that Amphotericin B was more effective than azoles against *Candida* isolates. However, other study done by Chakraborty et al (2017)⁽³¹⁾ found that non-*albicans* *Candida* was showing the high degree of resistance against Amphotericin B.

Conclusion

In present study candiduria was the most frequent form of candidiasis as 71% of *Candida* species were obtained from urine samples. *C. albicans* was the most predominant isolated species (63%), whereas non-*albicans* *Candida* species are increasing (37%). The high frequency of azole resistance especially Fluconazole among *Candida* species may reflect its increasing use as an empirical antifungal therapy, which may lead to the

appearance of resistant strains. The emergence of resistance could necessitate in-vitro drug susceptibility testing whenever antifungal treatment is described. On the basis of antifungal susceptibility profile of the isolates, Amphotericin B was the most effective antifungal agents that can be used against *Candida* species.

Limitations of the Study

One of limitation of this study is being conveniently sampled and hence may not represent the whole country. Species identification was based on phenotypic detection method which is less specific when compared to molecular methods.

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Conflict of Interests

The authors declared no conflict of interests.

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Original Article

Knowledge, Attitude, and Practice of Dental Students and Practitioners toward Noise-Induced Hearing Loss in Saudi Arabia: A Cross-Sectional Analytical Study

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Abstract

Background: Noise-induced hearing loss (NIHL) is a common occupational hazard for dental students and practitioners. Occupational safety laws are meant to protect them from occupational exposure to noise. However, adherence to these laws is determined by the level of knowledge, attitudes, and practice regarding NIHL.

Objectives: Limited number of studies has been conducted in this regards. Therefore, the present cross-sectional study was designed to assess Knowledge, Attitude, and Practice of Dental Students and Practitioners toward NIHL in Saudi Arabia.

Participants and Methods: This cross-sectional survey-based study of knowledge, attitudes, and practices regarding NIHL was administered & distributed through a link to a sample of 400 dental students and practitioners (out of a target population of 1000) from October 15 to November 15, 2019 in the Riyadh region, Saudi Arabia.

Results: A total of 252 participants (63% response rate) completed the survey; 55.6% were male and 44.6% female, with a mean age of 27.6 ± 8.47 , and 52.4% were students. Sample scores were weak for knowledge (4.48 ± 2.33) and practice (15.6 ± 5.97) and average for attitude (66.38 ± 7.62). Age, educational level, center designation, daily duration of exposure to occupational noise, nationality, and years of study & practice were statistically significant factors affecting scores of knowledge, attitude, and practice.

Conclusions: Dental students and practitioners in the Riyadh region scored weak to average on knowledge, attitude, and practice regarding NIHL.

Keywords: Noise-induced Hearing loss, Dental practice, Dental school, Dental instruments, Noise.

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Introduction

Noise-induced hearing loss (NIHL) is a common disease with a substantial economic, psychological, and social burden⁽¹⁻³⁾. NIHL is one of the most common types of sensorineural hearing loss & reported by the U.S. National Institute for Occupational Safety and Health (NIOSH) as avoidable and preventable⁽¹⁻³⁾; one of its most preventable causes is occupational exposure to noise⁽¹⁻³⁾.

Many developed countries have national laws that protect employees from unprotected exposure to noise and that oblige the employer to provide protective measures to employees and educate and train them about occupational NIHL⁽⁴⁾. However, for these laws to be effective, employees need to have a high sense of awareness and knowledge about occupational NIHL and attitudes and practices that match this knowledge in order to recognize these risks and the

importance of laws designed to mitigate them^(5,6).

Among the jobs where practitioners are exposed to high levels of noise are dentists. Dentists deal with occupational noise daily, especially from the use of high-speed dental drills^(1,7,8). Multiple studies have shown that dentists have a higher prevalence of NIHL compared to the general population^(1,9). In Saudi Arabia and, to the best of our knowledge, internationally, there is a lack of studies about knowledge, attitudes, and practice patterns among dental students and practitioners in regards to NIHL. This study aimed to assess knowledge, attitudes, and practice toward NIHL in the Riyadh region, Saudi Arabia.

Participants and Methods

Materials

This study used an analytical multicenter cross-sectional design. Using the PubMed database, an extensive literature review was conducted using the following keywords: noise-induced, hearing loss, dental. From the results, a

four-part online survey was designed following the survey designed and validated by Rus et al⁽¹⁰⁾. The first part of the questionnaire collected demographic data (age, gender, education level, years of experience, duration of daily work-related exposure to dental drills, and smoking status). The remaining three parts assessed the three domains focus of this study - knowledge, attitude, and practice. Rus et al⁽¹⁰⁾ reported Cronbach's α for knowledge, attitude, and practice as 0.67, 0.92, & 0.75, respectively, indicating internal consistency. Answers were selected from a predefined list for each item (Tables 1-3).

Sample

Power analysis for an independent sample t-test was conducted in G-POWER to determine a sufficient sample size using an alpha of 0.05, a power of 0.80, a medium effect size ($d = 0.35$), and two tails⁽¹¹⁾. Each group would have an equal allocation of participants. Based on these assumptions, the desired sample size was 194.

Table 1: Survey items, predefined list of answers, and participant response distribution for the knowledge domain. Data are presented as frequency distribution (and percentage) across the predefined list of answers for each item.

Item	Knowledge		
	Don't know	False	True
B1- Deafness due to noise is a more common problem among dental healthcare providers as compared to office workers	103 (40.9)	50 (19.8)	99 (39.3)
B2- Loud noise in dental clinics can cause hearing loss	86 (34.1)	86 (34.1)	80 (31.7)
B3- Hearing deteriorates when dental healthcare providers are exposed to hazardous noise	89 (35.3)	33 (13.1)	130 (51.6)
B4- Deafness can occur even if a worker is exposed to intermittent noise for a long period	95 (37.7)	53 (21.0)	104 (41.3)
B5- Hobbies like shooting and listening to loud music can cause deafness	50 (19.8)	32 (12.7)	170 (67.5)
B6- If people are exposed to noise, men are at higher risk of deafness than women	154 (61.1)	44 (17.5)	54 (21.4)
B7- Pus discharge from ear is an early sign of deafness due to exposure to loud noise	138 (54.8)	47 (18.7)	67 (26.6)
B8- Deafness due to noise can treated by taking medicine	116 (46.0)	84 (33.3)	52 (20.6)
B9- Deafness due to noise will recover to normal if a person is no longer exposed to excessive noise	100 (39.7)	46 (18.3)	106 (42.1)
B10- There is a law in Saudi Arabia that protects workers who are exposed to noise in the workplace	147 (58.3)	38 (15.1)	67 (26.6)
B11- It's the responsibility of the employer to provide ear plugs	97 (38.5)	37 (14.7)	118 (46.8)
B12- It's the responsibility of employees to wear ear plugs while working	74 (29.4)	37 (14.7)	141 (56.0)

Table 2: Survey items, predefined list of answers, and participant response distribution for the attitude domain. Data are presented as frequency distribution (and percentage) across the predefined list of answers for each item.

Item	Attitude				
	Neutral	Strongly Disagree	Disagree	Agree	Strongly Agree
C1- Dental healthcare providers will have deafness despite whatever preventive measures they use	81 (32.1)	56 (22.2)	81 (32.1)	26 (10.3)	8 (3.2)
C2- I'm not bothered by noise in the workplace	84 (33.3)	37 (14.7)	52 (20.6)	66 (26.2)	13 (5.2)
C3- Exposure to noise while working in dental clinics would not make me deaf	88 (34.9)	16 (6.3)	58 (23.0)	77 (30.6)	13 (5.2)
C4- I'm not worried if I can't hear properly after working in noisy places because it's only temporary	72 (28.6)	53 (21.0)	74 (29.4)	47 (18.7)	6 (2.4)
C5- Excessive exposure to noise can cause permanent deafness	85 (33.7)	14 (5.6)	26 (10.3)	102 (40.5)	25 (9.9)
C6- I'm not worried if my hearing starts to deteriorate	38 (15.1)	124 (49.2)	62 (24.6)	24 (9.5)	4 (1.6)
C7- I'll seek traditional medicine if I have start to experience deafness	65 (25.8)	27 (10.7)	44 (17.5)	79 (31.3)	37 (14.7)
C8- I don't need early treatment if I suspect deafness may be occurring because it's self-limiting	62 (24.6)	68 (27.0)	79 (31.3)	36 (14.3)	7 (2.8)
C9- I don't have to inform my employer if I have hearing loss	56 (22.2)	72 (28.6)	83 (32.9)	33 (13.1)	8 (3.2)
C10- Preventive measures toward deafness due to noise in dental clinics is important	61 (24.2)	7 (2.8)	5 (2.0)	104 (41.3)	75 (29.8)
C11- We should use ear plugs to avoid becoming deaf due to noise	88 (34.9)	10 (4.0)	23 (9.1)	90 (35.7)	41 (16.3)
C12- I like to use ear plugs	96 (38.1)	23 (9.1)	62 (24.6)	59 (23.4)	12 (4.8)
C13- Dentists must accept whatever type of ear plugs given to them	88 (34.9)	44 (17.5)	70 (27.8)	39 (15.5)	11 (4.4)
C14- Periodic audiometry assessment can detect deafness caused by noise in the workplace	123 (48.8)	6 (2.4)	11 (4.4)	92 (36.5)	20 (7.9)
C15- We should inform employers if a machine is noisier than before	59 (23.4)	6 (2.4)	9 (3.6)	107 (42.5)	71 (28.2)
C16- Training and health education for dental workers regarding methods on self-protection from noise should be done from time to time	80 (31.7)	5 (2.0)	14(5.6)	104 (41.3)	49 (19.4)
C17- Discussion with the dental practice employer regarding workplace noise will not reduce the occurrence of deafness from noise	100 (39.7)	16 (6.3)	70 (27.8)	55 (21.8)	11 (4.4)
C18- Only employers need to know occupational safety and health laws in detail	72 (28.6)	38 (15.1)	87 (34.5)	50 (19.8)	5 (2.0)
C19- Noise in the workplace is a usual thing for me	85 (33.7)	9 (3.6)	32 (12.7)	114 (45.2)	12 (4.8)
C20- It's easier to cover ears using fingers/hands rather than wearing ear plugs	61 (24.2)	72 (28.6)	94 (37.3)	21 (8.3)	4 (1.6)

Table 3: Survey items, predefined list of answers, and participant response distribution for the practice domain. Data are presented as frequency distribution (and percentage) across the predefined list of answers for each item.

Item	Practices			
	Never	Seldom	Frequently	Always
D1- I use ear plugs to protect my ears	184 (73.0)	39 (15.5)	20 (7.9)	9 (3.6)
D2- I undergo ear examinations by a doctor to detect deafness from noise	174 (69.0)	43 (17.1)	27 (10.7)	8 (3.2)
D3- I always use ear plugs when working	192 (76.2)	35 (13.9)	18 (7.1)	7 (2.8)
D4- I try to avoid noise as much as possible when I'm working	67 (26.6)	80 (31.7)	66 (26.2)	39 (15.5)
D5- When ear plugs are unavailable, I use whatever is available (e.g., cotton) to protect my ears from noise	180 (71.4)	34 (13.5)	26 (10.3)	12 (4.8)
D6- I discuss with my employers if ear plugs are broken	188 (74.6)	27 (10.7)	22 (8.7)	15 (6.0)
D7- Have you ever undergone an audiometry assessment?	169 (67.1)	48 (19.0)	30 (11.9)	5 (2.0)
D8- Has the employer arranged for their dental practitioners to undergo medical examination from time to time?	176 (69.8)	40 (15.9)	27 (10.7)	9 (3.6)
D9- Have you attended any seminar or course on deafness from noise?	197 (78.2)	29 (11.5)	21 (8.3)	5 (2.0)
D10- Has your employing institution conducted training on workplace health and safety?	140 (55.6)	47 (18.7)	46 (18.3)	19 (7.5)

Inclusion criteria were being a dental student, intern, resident, board-certified practitioner, and holding a MSc degree or PhD in a dental-related field in the Riyadh region of Saudi Arabia, as well as being currently active or employed and who agreed to participate voluntarily. Retired or unemployed dentists, dental nurses, those outside Riyadh region, and those who refused or withdrew consent were excluded.

Participants

A random number generator was used for simple random sampling to choose participants. The majority of the sample was Saudi, non-smokers, male dental students under 25 years old, with fewer than five years of experience, practicing in public tertiary centers and primary healthcare clinics, and with one to five hours of daily work-related exposure to dental drills (Table 4).

Informed Consent and Ethical Approval

Informed consent was obtained electronically from all participants, and the study protocol was approved by King Fahad Medical City IRB Number (IRB Log no. 20-256E)

Survey Procedure

Five volunteer administrators disseminated & distributed the survey through a link to a sample of 400 dental students and practitioners (out of a target population of 1000) in professional social groups in all five regions of Riyadh Province, Saudi Arabia. The survey ended on Nov 15, 2019. 252 participants completed the survey, representing a 63.0% completion rate.

Statistical Analysis

SPSS 26 software (SPSS, Inc., Chicago, IL, USA) was used to calculate descriptive statistics for each item. Inferential statistics were calculated using the Student's t-test and analysis of variance (ANOVA) to compare grouping variables for statistically significant differences for each domain's mean score. The Games-Howell test was used for post-hoc comparisons. A two-tailed p-value of 0.05 and Cohen's d values of <0.3, 0.3-0.5, and >0.5 were used to define statistical and clinical significance, respectively. Total score was calculated for each knowledge, attitude and practice domain. Then, each total raw score was transformed into 'percent score' by

dividing the score with highest possible score, and multiplying by 100. Base on that, a cut-off values below 50% was considered to be weak; 50-75 are average; >75% was high in adequate knowledge, attitude and practice.

Table 4: Participants' characteristics. Data presented as frequency distribution and percentage across all grouping variables (Demographic characteristics).

Variable		N	%
Age, years	<25	135	53.6
	25-30	55	21.8
	31-35	20	7.9
	>35	42	16.7
	Range (Mean \pm SD)	18 – 59 (27.63 \pm 8.468)	
Gender	Male	140	55.6
	Female	112	44.4
Education level	Student	132	52.4
	Intern	36	14.3
	Resident	30	11.9
	Specialist (= MSc)	27	10.7
	Board Certified (= PhD)	27	10.7
Years of study for students & interns (n = 168)	<1	25	14.9
	1-5.	125	74.4
	5-10.	18	10.7
Years of practice for dental practitioners (n = 84)	1-5.	33	39.3
	5-10.	15	17.9
	>10	36	42.9
Nationality	Saudi	214	84.9
	Non-Saudi	38	15.1
Center designation - students & interns (n = 168)	Tertiary	123	73.2
	Secondary	45	26.8
Center designation - dental practitioners (n = 84)	Tertiary	34	40.5
	Secondary	11	13.1
	Primary	21	25.0
	Private clinic	18	21.4
Working duration with dental drills (hours/day)	<1	18	7.1
	1-5	150	59.5
	>5	84	33.3
Smoking status	Never	193	76.6
	Ex-smoker	18	7.1
	Casual smoker	22	8.7
	Regular smoker	19	7.5

Results

For this study, the Cronbach's alpha coefficient for internal consistency was calculated for each domains. Cronbach's alpha value for knowledge, attitude, and practice was 0.73, 0.84, and 0.89, respectively. The result of the internal

consistency value was good in our study that means all the items in our survey were internally consistent and reliable. Regarding scores, overall, participants' scores were weak for knowledge (4.48 \pm 2.33) and practice (15.60 \pm 5.97) and average for attitude (66.38 \pm 7.62) (Table 5 and Figure 1).

Table 5: Overall scores for the knowledge, attitude, and practice domains. Data presented are frequency distribution and percentage for each grade level (weak, average, high) across all domains, along with the mean and standard deviations. A cut-off values below 50% was considered to be weak; 50-75 are average; >75% was high in adequate knowledge, positive attitude and good practice.

Variable	Knowledge		Attitude		Practice	
	N	%	N	%	N	%
Weak	174	69.0	3	1.2	203	80.6
Average	68	27.0	215	85.3	39	15.5
High	10	4.0	34	13.5	10	4.0
Total	252	100.0	252	100.0	252	100.0
Range	0 - 10		49 - 89		10 - 40	
Mean \pm SD	4.484 \pm 2.332		66.377 \pm 7.617		15.599 \pm 5.968	

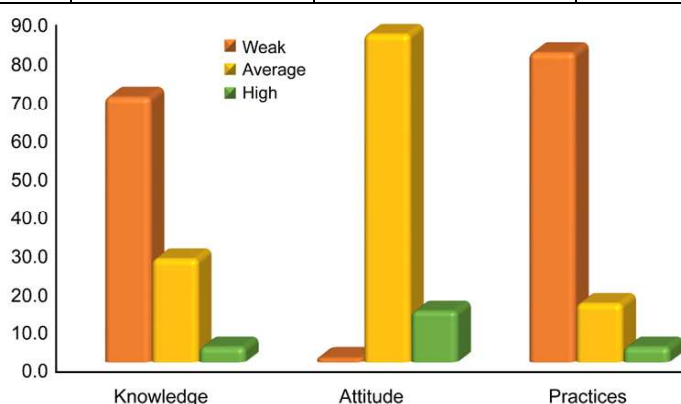


Figure 1: Overall scores for the knowledge, attitude, and practice domains. Data presented as percentage for each grade level (weak, average, high) across all domains.

The Knowledge Domain

Participants older than 35 years, master's or Ph.D. holders, non-Saudis, and had over 10 years of experience scored statistically significantly higher for knowledge than those who were younger than 35, students and residents, Saudis, and had fewer than 10 years of experience, with moderate to high clinical significance as evidenced by the effect size value of Cohen's *d* (Table 6). There were no statistically significant differences in knowledge scores between genders, center designations, daily exposure duration to dental drills, or smoking status (Table 6).

The Attitude Domain

Non-Saudis and master's degree holders scored higher in attitude than Saudis or students, interns, residents, and Ph.D. holders, and these differences were statistically significant, with high clinical significance (Cohen's *d* = 0.74 and 0.70,

respectively) (Table 7). Participants exposed daily to dental drills for more than five hours scored lower compared to those exposed fewer than five hours, which was statistically significant with moderate clinical significance (Cohen's *d* = 0.57) (Table 7). No statistically significant differences were found in attitude scores between age categories, genders, center designations, years of experience, or smoking status.

The Practice Domain

The differences among participants in practice scores regarding the grouping variables of age, gender, nationality, educational level, years of experience, center designation or smoking status were nonsignificant (Table 8). Participants with daily exposure to dental drills between 1 - 5 hrs scored lower in practice than those exposed fewer than one hr or >5 hrs daily; with a significant, with moderate clinical significance (Cohen's *d* = 0.41) (Table 8).

Table 6: Grouping variable scores for the knowledge domain. Data presented are the mean and standard deviations of age, gender, education level years of study for students & interns, years of practice for dental practitioners, nationality, center designation for students & interns, center designation for dental practitioners, duration of working with dental drills & smoking status. A type of statistical test (F: ANOVA for variables with more than two category, T: independent students t-test for variables with two category) used for comparison, test and p values, and effect size (ES; Cohen's d) for each level of each grouping variable. Primary healthcare center = PHCC.

Variable		N	Knowledge			F or T	ANOVA or t-test		
			Mean	±	SD		t	P	ES
Age, years	<25	135	4.311	±	2.149	F	10.348	<0.001	0.516
	25-30	55	3.582	±	2.307				
	31-35	20	4.900	±	2.426				
	>35	42	6.024	±	2.181				
Gender	Male	140	4.464	±	2.474	T	-0.151	0.880	0.019
	Female	112	4.509	±	2.152				
Education level	Student	132	4.394	±	2.189	F	7.869	<0.001	0.525
	Intern	36	3.528	±	2.384				
	Resident	30	3.733	±	1.946				
	Specialist	27	6.296	±	2.334				
	Board certified	27	5.222	±	2.259				
Years of study for students & interns	<1	59	3.949	±	2.232	F	0.980	0.377	0.157
	1-5.	98	4.286	±	2.215				
	5-10.	11	4.909	±	2.700				
Years of practice for dental practitioners	1-5.	33	4.121	±	2.132	F	9.214	<0.001	0.675
	5-10.	15	4.200	±	2.933				
	>10	36	6.222	±	1.884				
Nationality	Saudi	214	4.201	±	2.208	T	-4.769	<0.001	0.815
	Non-Saudi	38	6.079	±	2.398				
Center designation - students & interns	Tertiary	123	4.130	±	2.096	T	-0.743	0.458	0.122
	Secondary	45	4.422	±	2.650				
Center designation - dental practitioners	Tertiary	34	5.765	±	2.016	F	2.639	0.055	0.469
	Secondary	11	5.455	±	2.115				
	PHCC	21	4.429	±	2.908				
	Private clinics	18	4.111	±	2.272				
Working duration with dental drills (hours/day)	<1	18	4.667	±	2.401	F	0.368	0.692	0.082
	1-5	150	4.560	±	2.166				
	>5	84	4.310	±	2.607				
Smoking status	Never	193	4.570	±	2.322	F	0.442	0.723	0.110
	Ex-smoker	18	4.333	±	2.425				
	Casual smoker	22	4.273	±	2.142				
	Regular smoker	19	4.000	±	2.646				

Table 7: Grouping variable scores for the attitude domain. Data presented are the mean and standard deviation of age, gender, education level years of study for students & interns, years of practice for dental practitioners, nationality, center designation for students & interns, center designation for dental practitioners, duration of working with dental drills & smoking status. A type of statistical test (F: ANOVA for variables with more than two category, T: independent students t-test for variables with two category) used for comparison, test and p values, and effect size (ES; Cohen's d) for each level of each grouping variable.

Variable	N	Attitude			F or T	ANOVA or t-test		
		Mean	±	SD		t	P	ES
Age, years	<25	135	65.770	± 7.247	F	1.990	0.116	0.419
	25-30	55	66.436	± 6.428				
	31-35	20	70.200	± 7.557				
	>35	42	66.429	± 9.711				
Gender	Male	140	65.914	± 7.424	T	-1.078	0.282	0.136
	Female	112	66.955	± 7.847				
Education level	Student	132	65.970	± 7.278	F	4.013	0.004	0.683
	Intern	36	65.528	± 6.083				
	Resident	30	67.267	± 6.422				
	Specialist	27	71.185	± 8.540				
	Board certified	27	63.704	± 9.502				
Years of study for students & interns	<1	59	65.475	± 6.652	F	0.248	0.781	0.139
	1-5.	98	65.990	± 7.066				
	5-10.	11	67.000	± 8.978				
Years of practice for dental practitioners	1-5.	33	68.242	± 8.504	F	0.649	0.525	0.373
	5-10.	15	68.467	± 7.080				
	>10	36	66.139	± 9.384				
Nationality	Saudi	214	65.617	± 7.008	T	-3.862	<0.001	0.737
	Non-Saudi	38	70.658	± 9.419				
Center designation - students & interns	Tertiary	123	66.301	± 6.762	T	1.302	0.195	0.220
	Secondary	45	64.711	± 7.656				
Center designation - dental practitioners	Tertiary	34	65.853	± 9.169	F	0.615	0.607	0.439
	Secondary	11	68.727	± 8.900				
	PHCC	21	68.667	± 8.593				
	Private clinics	18	67.944	± 7.719				
Working duration with dental drills (hours/day)	<1	18	67.611	± 7.785	F	3.443	0.033	0.568
	1-5	150	67.213	± 7.265				
	>5	84	64.619	± 7.973				
Smoking status	Never	193	66.642	± 7.862	F	0.721	0.540	0.267
	Ex-smoker	18	63.944	± 6.941				
	Casual smoker	22	66.500	± 7.353				
	Regular smoker	19	65.842	± 5.852				

Table 8: Grouping variable scores for the practice domain. Data presented are the mean and standard deviation of age, gender, education level years of study for students & interns, years of practice for dental practitioners, nationality, center designation for students & interns, center designation for dental practitioners, duration of working with dental drills & smoking status. A type of statistical test (F: ANOVA for variables with more than two category, T: independent students t-test for variables with two category) used for comparison, test and p values, and effect size (ES; Cohen's d) for each level of each grouping variable.

Variable	N	Practice			F or T	ANOVA or t-test		
		Mean	±	SD		t	P	ES
Age	<25	135	16.007	± 6.709	F	2.076	0.104	0.402
	25-30	55	13.945	± 4.556				
	31-35	20	15.250	± 5.129				
	>35	42	16.619	± 5.108				
Gender	Male	140	15.214	± 5.684	T	1.145	0.253	0.144
	Female	112	16.080	± 6.298				
Education level	Student	132	15.803	± 6.155	F	1.230	0.299	0.348
	Intern	36	13.944	± 5.054				
	Resident	30	15.533	± 7.070				
	Specialist	27	15.296	± 3.841				
	Board certified	27	17.185	± 6.433				
Years of study for students & interns	<1	59	16.492	± 6.922	F	2.590	0.078	0.414
	1-5.	98	14.541	± 5.103				
	5-10.	11	17.273	± 6.842				
Years of practice for dental practitioners	1-5.	33	16.000	± 7.013	F	0.676	0.512	0.323
	5-10.	15	14.467	± 3.871				
	>10	36	16.611	± 5.693				
Nationality	Saudi	214	15.397	± 6.131	T	1.277	0.203	0.242
	Non-Saudi	38	16.737	± 4.870				
Center designation - students & interns	Tertiary	123	15.057	± 5.908	T	1.250	0.213	0.216
	Secondary	45	16.356	± 6.106				
Center designation - dental practitioners	Tertiary	34	16.382	± 5.805	F	1.126	0.343	0.514
	Secondary	11	14.091	± 2.844				
	PHCC	21	14.905	± 4.206				
	Private clinics	18	17.667	± 8.745				
Working duration with dental drills (hours/day)	<1	18	16.111	± 5.979	F	3.653	0.027	0.408
	1-5	150	14.787	± 5.047				
	>5	84	16.940	± 7.183				
Smoking status	Never	193	15.964	± 6.210	F	1.306	0.273	0.331
	Ex-smoker	18	15.444	± 4.829				
	Casual smoker	22	14.136	± 6.424				
	Regular smoker	19	13.737	± 2.725				

Discussion

The NIOSH recommends a maximum occupational noise level of 85 dB(A) for eight hours/day and has labeled NIHL as

the most common occupational hazard^(12,13). The occupational noise level in dental practice has been measured both internationally and nationally and was

found to be below this level^(7,14). However, other studies have shown that dental clinics are noise-polluted with levels that exceed the damage-causing level and for durations longer than eight hours⁽¹⁵⁻¹⁸⁾. Furthermore, multiple studies have shown NIHL's to be more prevalent among dentists compared to the general population^(1,9). The prevalence of NIHL in general population is 7-21%⁽¹⁹⁾. NIHL has a prevalence of 14% among dentists in Saudi Arabia and 16% internationally^(9,20,21). The Saudi Arabia Ministry of Labor has an occupational safety regulatory framework that mandates the employer to provide education, training, and protective equipment to employees to prevent occupational NIHL⁽⁴⁾. However, the adherence of employer and employees to these laws is predicated on a high level of NIHL awareness, its severity, employees' rights, employer's obligations, and the presence of nationally enforced laws in this regard.

Overall, participants' scores on knowledge, attitudes, and practice were alarmingly weak to average (Table 5). This is consistent with similar studies that have shown weak scores on knowledge and practice and average scores on attitude among workers occupationally exposed to noise, including dentists^(22,23).

Being younger (<35 years), with an educational level below board certification and fewer than 5-10 years of experience were associated with lower scores with statistical and clinical significance (Tables 6, 7), which might indicate an effect of education (i.e., the older, the more experienced, and the more educational achievements) on knowledge, attitudes. These findings are in agreement with similar studies showing a positive correlation between higher worker education level and higher knowledge, attitude, and practice scores^(12,24).

Dentists also tend to report NIHL as the least important work-related injury compared to work-related stress, vision problems, low-back pain, needle stick injuries, and allergies⁽²⁵⁾. This might be because of the lack of formal education on occupational safety regarding NIHL in particular. In this regard, to increase

awareness and education, an assessment of awareness is the first step⁽²⁶⁾. Educational interventions can include raising awareness about the prevalence and risk of NIHL in dental practice, protective measures at the personal and institutional levels, the necessity of regular personal audiometry assessment, the remedial and alternative options that might be offered if the audiometry assessment results are concerning, and employee rights and employer obligations within the Ministry of Labor's regulatory framework^(27,28). These educational interventions should be integrated into undergraduate and postgraduate curriculums, as undergraduates seem to be at high risk for NIHL based on their scores of knowledge and practice found in this study. This information can then be used to help inform regulatory and policymaking committees to improve policies and their enforcement.

This study is the first of its kind in Saudi Arabia and internationally. Because of its adequate sample size, it is a well-powered study, and its results provide critical insights by identifying at-risk groups for NIHL in a dental context so that appropriate remedial action can be taken. Future research should explore and design different educational interventions to improve knowledge, attitudes, and practice regarding NIHL among dental students and practitioners, measure these interventions' impact on adherence to occupational safety laws, and provide quality assurance of programs promoting occupational safety culture.

Conclusion

Dental students and practitioners in the Riyadh region scored weak to average on knowledge, attitude, and practice regarding NIHL. Leaders of dental education should design educational interventions to improve these aspects and integrate them into undergraduate and postgraduate curriculums. The current study's results can inform policymakers regarding measuring adherence of employers and employees to occupational safety laws in Saudi Arabia.

Limitations of the Study

Although the questionnaire used in this study was previously validated in several populations in its used form, as our participants are fluent in English, being not validated locally could be a little issue. Also, it was subject to the inherent limitations of survey designs (subjectivity, recall bias, and selection bias).

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Conflict of Interests

The author declared no conflict of interests.

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Original Article

Impact of Virtual Pathology Teaching Method on the Education of Medical Students at Qassim University – Saudi Arabia

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Abstract

Background: Only a few studies evaluated the outcomes of virtual pathology teaching for medical students.

Objectives: I aimed to evaluate the impact of virtual pathology teaching to medical students at Qassim University in comparison with the traditional light microscope-glass slide teaching method.

Participants and Methods: This study was conducted on exam records to assess the impact of the two methods of pathology teaching in two groups of medical students. The first included 2nd year students (n = 132; males = 88 with a mean age (\pm SD) of 18 ± 7.2 years, and, females = 44 with a mean age (\pm SD) of 17 ± 8.1 years), and, the second included 3rd year students (n = 135; males = 92 with a mean age (\pm SD) of 19 ± 8.7 years, and, females = 43 with a mean age (\pm SD) of 18 ± 5.1 years). Exam performance was checked for each of the two groups after receiving pathology teaching through both of glass slide and virtual slide methods. The two tests were separated by an interval period. Intra-group and intergroup comparisons were made. A six-close-ended-questions questionnaire asking for opinion concerning microscopic traditional vs. virtual histopathology teaching was distributed to the students and results were assessed.

Results: The performance of the 1st group in the glass slide exam was poor with a failure rate of >60%, while their performance was good in the virtual slide exam; >75% of them get ≥ 8 out of 10 marks and only 17% of the students failed the exam. The 2nd group includes 267 students. The performance of the 2nd group in the glass slide exam was excellent as >82% of them get excellent marks with only 6% failure rate, while, the failure rate was higher in their virtual slide exam. Analysis of the questionnaire showed that most of the students (62.5%) prefer virtual pathology in general because it had an easy accessibility and it saved their studying time, in contrast to the glass slide. However, still some of them prefer the use of both methods together in learning.

Conclusions: 62.5% of the medical students preferred virtual pathology studying method with the supplementation of traditional light microscope teaching method. However, the impact of virtual slide teaching method for pathology on medical students' learning needs further studying.

Keywords: Light microscope, Pathology glass slide teaching, Pathology virtual slide teaching, Medical students, Qassim, Saudi Arabia.

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Introduction

As in any other aspects of our life, computer and technologies had been introduced to many educational fields including pathology as electronic books,

videos, audios, virtual slides, etc. Around the mid-1980s, virtual microscopy and virtual histopathology had been developed for the first time⁽¹⁻³⁾, but it was not

practically used due to financial and technical problems, like the high cost of the computers and their low speed processors & limited memory. In the beginning of the 2000s, computers become cheaper with large memories and faster processors, which empowered virtual pathology to be introduced into both educational as well as diagnostic purposes^(1,2). Because of this, many medical schools in the world (like in Saudi Arabia, USA, Switzerland, Germany, Poland, Australia, Turkey, Taiwan, Mozambique, Serbia, etc.)⁽¹⁻⁶⁾ started to implement the virtual pathology in teaching of pathology courses as a new tool with the potential to replace the usual traditional glass slide teaching method. At College of Medicine, Qassim University, Saudi Arabia, the histopathology practical sessions are taught by using a combination method of both glass slides and virtual slides at equal proportions (50%). However, this ratio may vary depending on the availability of a good quality glass slides.

The aim of this study was to evaluate the impact of utilizing the virtual slide pathology teaching method for the medical students (based on both knowledge and skills) at College of Medicine, Qassim University, Saudi Arabia, in comparison with the traditional light microscope-glass slide teaching method.

Participants and Methods

Setting and Design

This study was conducted on exam records of the 2nd and 3rd years' medical students of the basic phase of medical education, College of Medicine, Qassim University, Buraydah, Qassim, Saudi Arabia, in the academic years 2018 and 2019. De-identified data from results of two consecutive traditional then virtual exams for same group of 2nd year students, and, from a traditional or a virtual exam for two different groups of 3rd year students, plus a questionnaire assessment of the students' opinion concerning the two approaches of histopathology teaching were utilized. This study was ethically approved by

Qassim University Ethical Committee (Approval #1441-975795).

Paired and unpaired assessment of the methods of histopathology teaching (traditional vs. virtual slides)

In the first paired part of this study, the impact of teaching histopathology using virtual slides in comparison with the traditional glass slides was evaluate. We analyzed exam records of one group of 2nd year students' who previously underwent two consecutive histopathology exams for each method. This group was composed of 132 students (males $n = 88$ with a mean age (\pm SD) of 18 ± 7.2 years, and, females $n = 44$ with a mean age (\pm SD) of 17 ± 8.1 years). Each student was asked to provide a diagnosis and a histopathological description of a traditional glass slide, which was part of a final practical exam that was conducted in December 2018. The recoded exam results were referred to as Results-S1; where "S" indicates the same group of students and "1" indicates the first set of exam record data. Four months later, the same group of students was tested again and each student was asked to provide a diagnosis and a histopathological description of a virtual slide as part of a final practical exam. The results of the later exam were recoded and referred to as Results-S2, where "2" refers to the second set of exam record data. The S1 vs. S2 results were statistically compared to explore the impact of the used method of teaching; virtual vs. traditional glass slides on students' exam performance. Both of the traditional glass slide (that was provided in the earlier exam) and the virtual slide (that was provided in the later exam) were for the same disease with classical histopathological morphology (granuloma). However, the slides themselves were different to avoid any memorization effect that could affect the quality of the analysis.

In the second unpaired part of this study, we enrolled two different subgroups (A and B) of 3rd year medical students. 3rd year group A was composed of 135 students (males $n = 92$ with a mean age (\pm SD) of 19 ± 8.7 years, and, females $n = 43$ with a mean age (\pm SD) of 18 ± 5.1 years). Each student was asked to provide

a diagnosis and a histopathological description for a traditional glass slide as part of a final practical exam conducted in December 2018. The results were recoded and referred to as Results-D1; where "D" indicates a different group of students and "1" indicates the first set of exam record data of group A. Group B included 132 students (86 males $n = 86$ with a mean (\pm SD) of 18 ± 7.2 years, and, females $n = 46$ with a mean age (\pm SD) of 17 ± 8.1 years). Each student was tested using a virtual slide, which was part of a final practical exam that was conducted in December 2019. The results were recoded and referred to as Results-D2, where "2" indicates the second set of Group B exam record data. D1 and D2 results were statistically compare to assess the impact of the methods of teaching used on the students' exam performance. The virtual

slide and the traditional slide were for the same disease with classical histopathological morphology (clear-cell renal cell carcinoma), however the slides themselves were different.

For the light microscope, we use Olympus microscope model CX21 that is equipped with x4, x10, x20, and x40 objective lenses. To scan the glass slides for preparing the virtual slide copies, we use Aperio (from Leica) to scan these slides at different powers x4, x10, x20, and x40 using ImageScope software to visualize these slides.

A six-close-ended-questions questionnaire (Figure 1) asking the students' opinion concerning microscopic traditional vs. virtual histopathology teaching was distributed and its results were analyzed.

Question	Light Microscope	Virtual Slide
Which of the following two educational methods is <i>easier to gain an access to</i> ?		
Which of the following two educational methods does <i>save more time during studying</i> ?		
Which of the following two educational methods is <i>easier to deal with for studying</i> ?		
Which of the following two educational methods is <i>easier to navigate and handle</i> ?		
Which of the following two educational methods is <i>has clearer and higher quality images</i> ?		
In general, which of the following two educational methods for histopathology do <i>you prefer using</i> ?		

Figure 1: The distributed six-close-ended-questions questionnaire asked for student opinion concerning microscopic traditional vs. virtual histopathology teaching.

Statistical methods

Statistical significance of comparisons was evaluated by Student's t-test. P values of ≤ 0.05 were considered significant. All statistical analyses were done by using statistical package for social sciences (SPSS) version 14.0 for Windows (Chicago, IL. USA).

Results

The summary of the first part of paired impact assessment for virtual slide vs. traditional glass slide teaching of histopathology for 2nd year students is demonstrated in Table 1 and Figure 2. The performance of this group for the

glass slide exam was evidently poor ($p < 0.05$ vs. virtual side performance) since more than 45% of the students gain ≤ 2 out of 10 marks and only $\frac{1}{3}$ of them gain the pass mark (≥ 6 out of 10 marks). In the later virtual side exam (conducted 4 months later), the students' performance was markedly improved since $>75\%$ of them gain excellent marks (≥ 8 mark out of 10 marks). This is a significant achievement for the virtual teaching approach. However, this is also could have been influenced by the build-up of higher experience in the analysis & interpretation of the histopathology slides

during 4 months period of studying with the more easily accessible virtual slides.

Table 1: Exam performance for glass vs. virtual slides histopathology teaching paired comparison by the same group of 2nd year medical students (n = 132); 4 months apart. Data shown are frequency; n (%) and p value.

Slide Type	Paired Histopathology Exam Mark Distribution					P
	0 to <2	2 to <4	4 to <6	6 to <8	8 to 10	
Glass Slide	62 (46.97)	13 (9.85)	10 (7.58)	5 (3.79)	42 (31.82)	<0.05
Virtual Slide	15 (11.36)	4 (3.03)	5 (3.79)	8 (6.06)	100 (75.76)	

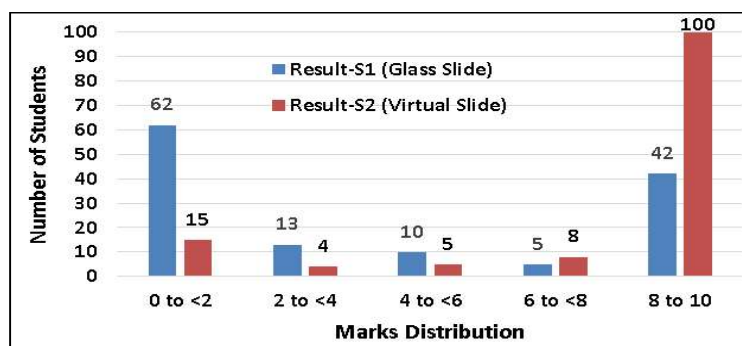


Figure 2: Paired comparison of glass vs. virtual slides teaching impact on histopathology exam performance (Marks out of 10) for the same groups of 2nd year medical students. Data shown are frequency; n.

The summary of the second unpaired part of this study assessing impact of virtual slide vs. traditional glass slide teaching of histopathology for the two different groups of 3rd year students is presented in

Table 2 and Figure 3. Those with the glass slide teaching method revealed significantly better performance than the virtual one ($p < 0.05$).

Table 2: Exam performance for glass vs. virtual slides histopathology teaching unpaired comparison by the two different groups of 3rd year medical students. Data shown are frequency; n (%) and p value.

Type of Exam	Unpaired Histopathology Exam Mark Distribution					P
	0 to <2	2 to <4	4 to <6	6 to <8	8 to 10	
Glass Slide, n = 135	6 (4.44)	1 (0.74)	2 (1.48)	14 (10.37)	112 (82.96)	<0.05
Virtual Slide, n = 132	15 (11.36)	1 (0.76)	4 (3.03)	9 (6.82)	103 (78.03)	

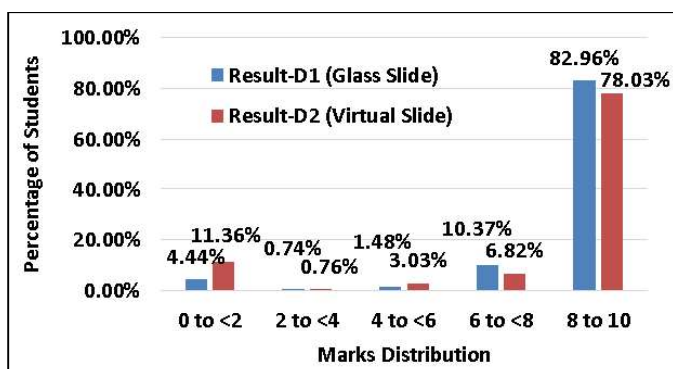


Figure 3: Unpaired comparison of glass vs. virtual slides teaching impact on histopathology exam performance (Marks out of 10) for the two different groups of 3rd year medical students.

In general, the exam performance of 3rd year students was markedly better than 2nd year students ($p < 0.05$) after both of glass and virtual slides method of the teaching since $>70\%$ of them gained excellent marks (≥ 8 out of 10 marks). This might be due to 3rd year students being more experienced than the 2nd year students.

The summary of questionnaire results concerning the opinion of students toward microscopic traditional vs. virtual

histopathology teaching is demonstrated in Figure 4. Students slightly preferred virtual slides compared to glass slide teaching (62.5% ; $p > 0.05$); since it has an easy access (93.75%), save studying time (100%), and easier to deal with and handle (87.5%). However, the students admitted that light microscope and glass slides have better quality of image (87.5%) and easier to be navigated (90.63%).

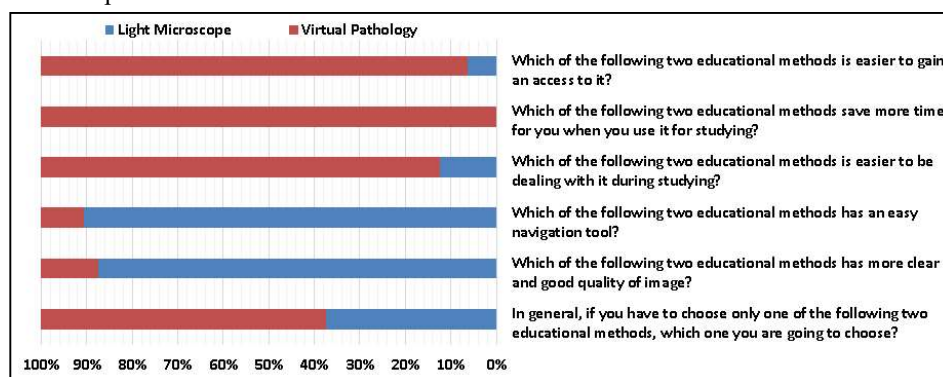


Figure 4: Results of students' answers regarding the items of the distributed questionnaire that compared their opinion concerning microscopic traditional vs. virtual histopathology teaching.

Discussion

Education of pathology needs combination of basic principles & knowledge supported by practical training sessions to clarify these principles in a visual form rather than leaving this for the imagination of the students⁽²⁾. Therefore, linking between principles of disease pathogenesis, gross morphology of the organs/lesions, histopathological features of the tissue, and the clinical pictures is essential when teaching pathology courses. Light microscope was the only available and widely used tool in the practical teaching courses of pathology⁽²⁾, but after the year 2000 and with the advent of virtual pathology teaching, the role of light microscope was re-evaluated by many teachers and institutions (including our institution)^(1-3,7-15). Some instructors suggested reforming the teaching method of pathology courses by replacing the traditional light microscope practical sessions by virtual pathology^(16,17). Accordingly, virtual pathology turned to play an increasing role in teaching pathology educational courses for both of undergraduate and

postgraduate students⁽¹⁾. In our medical institution, we officially implemented virtual pathology as a supportive educational method for histopathology since 2013.

The performances of the students in virtual slides exams were better or at least equal to their performances in the traditional glass slides exams. This result suggest that virtual pathology can replace the current traditional glass slide teaching method in the teaching process of the undergraduate medical students at least without affecting their performances. This idea is supported by many other researchers. A research group from University of Minnesota, USA studied the performance of the medical students in the hematopathology courses by using virtual pathology teaching method in one group and traditional glass slide method in the other group⁽¹⁸⁾. They found that the virtual microscopy group performed significantly better than the traditional microscopy group. Another research done in Tabuk University, KSA showed that the students' performances in both MCQs and OSPE in virtual microscopy was

better than in light microscopy⁽⁴⁾. A Brazilian research group conducted a research on undergraduate dental students to evaluate their performances in oral pathology courses by using virtual microscopy in contrast to conventional microscopy⁽¹⁹⁾. They reported that students' good performances on the knowledge exam suggest that virtual pathology teaching technology represent an important tool in oral pathology teaching in dental education. Not all the authors agree on this matter. For example, Scoville and Buskirk compared these two methods experimentally in a classroom setting and found that the performance of the students in the exams was not significantly influenced by the teaching method used⁽²⁰⁾.

One of the major factors that affect the performance of students is the experience in using virtual pathology. In our results, it is clearly that the performance has a direct relationship with the experience of the students in both pathology (as knowledge) and using virtual slides (as a skill). Therefore, the marks of 3rd year medical students group are much better than 2nd year group. This conclusion is supported by many other researches. A research group in Taiwan conducted a research on the academic performance of undergraduate students in histology and pathology courses⁽²¹⁾. They found that the student performance is positively associated with their prior experience in using the virtual pathology. Ordi and his research group from Barcelona, Spain evaluate the use of virtual microscopy in the undergraduate teaching of pathology⁽¹⁷⁾. They concluded that students' experience with virtual microscopy is very favorable. Another publication by Kuo and Leo from Canada conclude that it is critical to ensure trainees acquired adequate experience to competently operate virtual microscopy so performance would not be affected by non-content factors⁽²²⁾. Moreover, some authors suggest that virtual pathology can effectively replace the traditional methods of pathology teaching⁽¹⁷⁾. Others still believed that although virtual pathology method is a good tool in teaching pathology, still there is a need for the traditional microscopy method in the

teaching process of pathology and its should not be neglected^(19,23). In our survey, still 34.5% of our participating students favored traditional microscopy teaching method, mainly due to its better quality of image.

According to the results of our survey, the majority of the students whom participate in the questionnaire favored virtual pathology as a studying method because of its easy accessibility and handling. The same result was obtained in a research done by a group of researcher in Universidad de Los Andes, Santiago, Chile⁽²⁴⁾. Also in another survey done by Ordi et al in Spain, the majority of the students found that virtual pathology is easier to be navigated and used in comparison with traditional microscopy⁽¹⁷⁾. Another finding that should be highlighted in our survey is the time management. All the participating students admit that virtual pathology is a time-saving tool, mainly because of its easy accessibility and availability anytime anywhere. The same finding was found also by many other researchers^(17,19).

Virtual pathology has many advantages over the light microscope; like easy accessibility and handling. However, it is still has some disadvantages; like its image low resolution^(1,17,25-28). So here, I will discuss some of the comparable points between virtual and traditional teaching methods of pathology (summarized in Table 3). In comparison with light microscope, virtual pathology has relatively low cost on the long-term. However, both required prepared histopathological glass slides to be examined in the former and scanned for the latter. Afterwards, maintaining the glass slide and the microscope is no longer needed for the virtual pathology. Moreover, sharing and copying virtual slides can be easily done between many students, institutions, and countries, and can be commercially purchased⁽¹⁾. For example, our institution shares all its virtual pathology resources with College of Medicine, Shaqra University, Shaqra, Saudi Arabia. Such feature is almost impossible with the light microscope and glass slides.

Table 3: Comparison between traditional glass slides, virtual sides, and pictures as teaching methods for histopathology^(1,17,25-28).

Feature	Glass Slide	Virtual Slide	Picture
Costs	Expensive	Intermediate	Cheep
Maintenance	Expensive and time consuming	Cheep	Cheep
Accessibility of the students for the educational material	Limited to specific time and place	Unlimited, needs only internet and computer/smart device	Unlimited, needs only internet & computer/smart device
Quality and clarity	Clear	Mostly clear	Variable
Interactive	Yes	Yes	No
Looking at the whole slide	Yes	Yes	No
Focusing issues	Best	Intermediate	Poor
Refractive Index testing	Best	Intermediate	Poor
Magnifications	Excellent	It has some limitation on both screening view and high-power view	Variable
Saving the material	Need storage room	Needs hard disc with big memory	Needs hard disc with big memory
Copying the material with the same exact morphology	Limited	Unlimited	Unlimited
Adding comments on the material	Limited	Unlimited	Unlimited
Light and illumination adjustment	Complex process that must be trained person	Automatically done, so no need for experience	Automatically done, so no need for experience
Looking by using both eyes	Difficult for the beginners	Easy from the beginning	Easy from the beginning

Moreover, glass sides are breakable, spoilable, their stain is bleachable and require specific storage conditions of light, humidity and temperature. Such maintenance and renewal costs money and human technical resources, all are almost dispensable for virtual histopathology teaching^(1,2). Furthermore, virtual pathology sides are accessible and usable anywhere and at any time needed, using a computer or a smart device provided with the reading software and a soft copy of the virtual slide. Virtual sides are even accessing over the internet (as what we do at our college)^(1,2,13-15). This has a positive impact on histopathology education, since students are no longer in need to be present physically at specific place and at a specific time to get access for learning or reviewing^(2,13-15). This was invaluable in the lockdown time of the newly emerging Covid-19 pandemic. These features are not applicable to glass side-light microscope teaching methods. This saves time and improves preparedness for exams and performance;

as it was clear from the present study results - particularly with students with basic experience of pathology. Another advantage of virtual pathology is duplication and sharing the exact same slide between hundreds and thousands of students/institutions⁽¹⁾. So, any slide with classical morphology or very rare lesion can be reproduced and distributed. This cannot be done with glass slides since multiple recurs from the same paraffin block can diminish the lesion areas as well as the entire tissue. This may ends up with many glass slides of different morphology⁽²⁾. Therefore, to make a good set of teaching glass slides, all of the recuts must be costly revised by an experienced pathologist to make sure that they are adequate, clean, clear, and content-unified for teaching⁽²⁾.

It is also important to point out technical issues which were very much similar to the technical issues arises in the previously published studies in term of light adjustment, focusing, looking by both eyes, magnifications, refraction, and

labelling^(1,2,13-15). However, manipulating the focus to visualize some unclear/hiding histopathological features cannot be done with the fixed-image of virtual slides, in contrast to light microscope⁽¹⁾. Moreover, using both eyes for examination of glass slides was difficult for the students using the light microscope. But with the time, they gain the skill and start to visualize the things under the microscope by using both of their eyes. In addition, magnification is another issue with virtual side teaching particularly at low-power and x100 high-power view with some limitations in resolution, since both of these magnifications are usually not clear on the screen⁽¹⁾. In contrast, the resolution of these two powers is excellent when using the light microscope. Moreover, testing the refractive index of the light microscopic image is diagnostically advantageous; this can be done only by using the light microscope⁽¹⁾. Furthermore, labelling the slides with illustration was found to be easier for the virtual pathology. This is very helpful for the teaching process of the students especially during the self-directed learning^(1,2).

However, there is no clear cut answer to the question: Can virtual pathology teaching method replace light microscope teaching method? This is a controversial issue among experts. Some authors claim that histopathology teaching using virtual slides has the same efficacy of light microscopic teaching and can easily replace it^(2,4,17,26-29). Others insist that light microscope teaching method must be used to allow the students gain real life skills, whereas virtual pathology can be used as a supplementary educational tool^(24,30). In support, 37.5% of our study students admit that light microscopic teaching is an essential part for histopathology educational process.

Conclusion

Virtual histopathology teaching method for the undergraduate medical students is still a controversial issue among experts, but 65.5% of the studied medical students preferred virtual pathology as a teaching method with the supplementation of traditional light microscope teaching method. In general, there is a positive

impact of the virtual pathology on the students' performance. However their performance is influenced by their experience in dealing with this method of teaching as students with more experience, with glass side teaching, tend to perform better. Therefore, I recommend starting with traditional glass slide teaching method, and then shifting gradually to the virtual pathology. However, the value of using of virtual slide teaching method for pathology for medical students needs further studying that should get to solutions for its known limitations.

Limitations of the Study

This study has few limitations, teaching by virtual slides is not limited to the histopathology only as it is used by many other disciplines like hematopathology, cytology, histology, and microbiology^(1,7,8,17,26-29). Exam performance for these disciplines and comparisons were not considered in this study.

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Conflict of Interests

The author declared no conflict of interests.

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4. Duplicate publication, sometimes called self-plagiarism, occurs when an author reuses substantial parts of his or her own published work without providing the appropriate references. This can range from getting an identical paper published in multiple journals, to 'salami-slicing', where authors add small amounts of new data to a previous paper. Self-plagiarism, also referred to as 'text recycling', is a topical issue and is currently generating much discussion among editors. Opinions are divided as to how much text overlap with an author's own previous publications is acceptable. We normally follow the guidelines given in COPE website. Editors, reviewers and authors are also requested to strictly follow this excellent guideline (Reference: Text Recycling Guidelines: <http://publicationethics.org/text-recycling-guidelines>).
5. In case of "suspected minor plagiarism", authors are contacted for clarification. Depending on all these reports, reviewers and editors decide final fate of the manuscript.
6. Use of automated software is helpful to detect the 'copy-paste' problem. All submitted manuscripts are checked by the help of different databases, eTBLAST, Plagiarism Detection tools, etc. At the same time scientific implication of the case ('suspected minor plagiarism'), also judged by reviewers and editors. Plagiarism Detection tools are useful, but they should be used in tandem with human judgment and discretion for the final conclusion. Therefore, suspected cases of plagiarisms are judged by editors on 'case-to-case basis'.
7. Editors have the final decision power for these cases.

Ethics in publishing

Policy and ethics

The work described in your article must have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans (<http://www.wma.net>); Uniform Requirements for manuscripts submitted to Biomedical journals (<http://www.icmje.org>) published by the International Committee of Medical Journal Editors. An official local authorized body should review the research project before its beginning and a document acknowledging the ethical clearance of the research could be requested from prospective authors. This must be stated at an appropriate point in the article (Material and Methods). Research papers based on animal studies should get a similar ethical clearance from an official committee for the animal welfare.

Cover letter

A cover letter is required to accompany the manuscript submission along with the Manuscript Submission/Copyright Transfer Form. It should include information about the following points relevant to the specific type of your article:

- Why should JUMJ publish your manuscript?
- Relevance to JUMJ publication policy.
- Potential competing interests.
- Approval of the manuscript by all authors.
- Adherence to Simultaneous and Duplicate Publication Policy.

Conflict of interest

All authors are requested to disclose any actual or potential conflict of interest including any financial, supplements, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form,

in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by an appropriate originality detection service.

Authorship

All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts before the accepted manuscript is published in an online and/or printed issue.

- Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement.
- In the case of addition or removal of authors, this includes confirmation from the author being added or removed.
- Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above.
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- After the accepted manuscript is published in an online and/or printed issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement, then this should be stated.

Open access

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The processing and publication fee

No processing or publication fee is required.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use any English Language Editing service available. The abstract content will be translated into Arabic to accompany the published manuscript as an Arabic Abstract. In case the author's mother language is not Arabic, the Journal will help preparing it.

Submission

Manuscript submission and follow up to this journal proceeds totally online through email communications (ajms@ju.edu.sa). Complete manuscript with tables and figures inserted within the text at their final place should be submitted as a single file in the two; word and PDF formats. The submission and copyright transfer form is available on request and is mandatory to hand fill, sign and date by all authors before any processing of the submitted material. The form is provided at the last page of every issue of JUMJ. Authors are encouraged to print and fill the form and submit alongside with the manuscript.

Referees

A minimum of six suitable potential reviewers (please provide their name, email addresses, title, institutional affiliation, and, ORCID or Scopus ID). When compiling this list of potential reviewers please consider the following important criteria: They must be knowledgeable about the manuscript subject area; must not be from your own institution; at least two of the suggested reviewers should be from another country than the authors'; and they should not have recent (less than four years) joint publications with any of the authors. However, the final choice of

reviewers is at the editors' discretion. *Excluding peer reviewers:* During submission you may enter details of anyone who you would prefer not to review your manuscript.

Types of submission and criteria

Original Research Communications may be offered as Full Papers or as Short Communications. The latter format is recommended for presenting technical evaluations and short clinical notes, comprising up to 1,500 words of text, 15 references, and two illustrative items (Tables and/or Figures).

Case Reports will be accepted only where they provide novel insight into disease mechanisms, diagnostic, and management applications.

Critical Reviews will be welcome but prospective authors are strongly advised to seek authorization from the Editor-in-Chief to avoid conflict with scheduled reviews invited by the Editorial Board. They should address new topics or trends in fields of the Journal Scope.

Editorial and opinion pieces Please contact the Editor-in-Chief for consideration.

PREPARATION

NEW SUBMISSIONS

Submit your manuscript with single-spaced text as a single PDF file and a single Word document file, in any format or layout that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing.

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), chapter title/article title, journal title/book title, year of publication, volume number-issue number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements

On initial submission, there are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript message; Title, Abstract, Keywords, Introduction, Materials/Patients and Methods, Results with Artwork, Figures and Tables with legends and titles (below the figure and on top of the table, respectively), Discussion, Limitations of the

study and Future directions, Gain of Knowledge, Conclusions, Conflict of Interest, Acknowledgement (if any), and References. Upon final acceptance, the author(s) will be instructed to reformat their manuscript according to JUMJ format detailed below.

If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.

Divide the article into clearly defined sections with title, subtitles and sub-subtitles on separate lines whenever applicable.

Figures and tables embedded in text. Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript.

All standard and non-standard abbreviations should be defined in full at the first mention in the text and should be consistent throughout the paper.

In the initial submission, it is advisable to have references in names (e.g., Smith et al, 2014) within the text rather than numbering them. Revision and correction frequently necessitate dropping or inserting text with their references. Numbering references in that stage will create the problem of renumbering them in the text and list.

ORIGINAL RESEARCH PAPER WRITING TEMPLATE

Papers include original empirical data that have not been published anywhere earlier or is not under consideration for publication elsewhere (except as an abstract, conference presentation, or as part of a published lecture or academic thesis), and after accepted for publication it will not be submitted for publication anywhere else, in English. Null/negative findings and replication/refutation findings are also welcome. If a submitted study replicates or is very similar to previous work; authors must provide a sound scientific rationale for the submitted work and clearly reference and discuss the existing literature. Submissions that replicate or are derivative of existing work will likely be rejected if authors do not provide adequate justification. Studies, which are carried out to reconfirm/replicate the results of any previously published paper on new samples/subjects (particularly with different environmental and/or ethnic and genetic background) that produces new data-set, may be considered for publication. But these types of studies should have a 'clear declaration' of this matter. The English language in submitted articles must be clear, correct, and

unambiguous. No limits for the total number of words for articles of this type.

Title page information

Page 1 of the typescript should be reserved for the title, authors and their affiliation and addresses.

Title. Concise, informative and reflects the study content. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

Running Title: A shorter running title of no more than 55 letters including spaces should be provided.

Author names and affiliations. Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a superscript Arabic number immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and the e-mail address and phone number (with country and area code) of each author.

Corresponding author. The corresponding author should be indicated in addition with a superscript asterisk * immediately after his/her affiliation superscript Arabic number. The corresponding author will handle correspondence at all stages of refereeing, publication, and post-publication. Contact details must be kept up to date by the corresponding author.

Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript lower-case letters are used for such footnotes.

Abstract

Page 2 of the typescript should be reserved for the abstract which should be presented in a structured format and should not exceed 350 words. The following headings should be included for research articles followed by a colon: a) Background, b) Hypothesis/Objectives: c) Materials/Patients and Methods: d) Results: e) Conclusions (should be data justified). Suitable headings could be used for other types of publications (Case reports, Review articles, etc.).

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major

conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided. Non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Keywords

Immediately after the abstract, provide a maximum of 10 keywords for full papers, or 5 keywords for Short Communications, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, "and", "of"). Please use terms from the most current issue of medical subject headings of Index Medicus. The key words should cover precisely the contents of the submitted paper and should give readers sufficient information as to the relevance of the paper to his/her particular field. Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Introduction

Provide adequate background that highlights the importance and gap information of your research point in relation to previous studies but avoiding a detailed literature survey. State the hypothesis or rationale and objectives of the work and a brief description of how you planned to approach them.

Materials or Patients and Methods

Provide sufficient detail to allow the work to be reproduced, with details of supplier and catalogue number when appropriate. Methods already published should be indicated by a reference: only relevant modifications should be described.

Patients and Normal Subjects

If human participants were used in the experiment please make a statement to the effect that this study has been approved by your Institution Ethics Review Board for human studies (the number of the approval should be stated in the methods section and JUMJ may ask for submission of the original ethical approval with the manuscript), and, that patients or their custodians have signed an informed consent that also states right of withdrawal without any consequences. Sample sized should be appropriately calculated. The manuscript should describe how the size of the experiment was planned. If a sample size calculation was performed this should be reported in detail, including the expected difference between groups, the expected variance, the planned analysis method, the desired statistical power and the sample size thus calculated. For parametric data, variance

should be reported as 95% confidence limits or standard deviations rather than as the standard error of the mean. Normal participants and patients criteria, inclusion and exclusion criteria should be stated. Name and address where the work was done and when it was done (time period, from to) should be clearly stated, too.

Experimental animals

When animals were used in the experiments, a local Institutional Ethics Review Board for animal studies should review and approve the experiment and that all animal procedures were in accordance with the standards set forth in guidelines for the care and use of experimental animals by Committee for Purpose of Supervision of Experiments on Animals (CPCSEA) and according to National Institute of Health (NIH) protocol. The precise species, strain, sub-strain and source of animals used should be stated. Where applicable (for instance in studies with genetically modified animals) the generation should also be given, as well as the details of the wild-type control group (for instance littermate, back cross etc.). The manuscript should describe the method by which animals were allocated (randomized) to experimental groups, particularly for comparisons between groups of genetically modified animals (transgenic, knockout etc.), the method of allocation to for instance sham operation or focal ischemia should be described.

Experimental

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described. Where and when the study was conducted should be stated.

Results

Results should be clear and concise. Data should be presented in an appropriately organized tables, figures and/or artworks. The statistical analysis used should be suitable for the objectives of the study and type of data analyzed. Prospective authors are highly advised to consult a biostatistician.

Footnotes

Footnotes should be used sparingly. For table footnotes, indicate each footnote in a table with a superscript lowercase letter or add them into the title (preferable).

Graphical abstract

A Graphical abstract is optional and should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors

must provide images that clearly represent the work described in the article. Please provide an image with a minimum of 531×1328 pixels ($h \times w$) or proportionally more. The image should be readable at a size of 5×13 cm using a regular screen resolution of 96 dpi. It is preferable to be inserted at its normal place to the relevant text or otherwise be submitted as a separate TIFF, EPS, PDF or MS Office files.

Discussion

This should explore the significance, interpretation and reasoning of the results of the work vs. other studies. Do not repeat describing the results in this section. A combined Results and Discussion section is acceptable. Avoid extensive citations and discussion of published literature. In the same time, avoid speculations without a supporting literature. Avoid discussion based on "Data not Shown" or "Personal Communications".

Limitations and Future Prospective

The authors may wish to pinpoint the limitations of the study and their reason and foresee the next step to go from their study. This may be presented in a short Limitations and Future Prospective section standing alone or as a separate paragraph in the Discussion or Results/Discussion section.

Conclusions & Gain of Knowledge

The main conclusions of the study may be presented in a short Conclusions section standing alone or as a separate paragraph at the end of the Discussion or Results/Discussion section. Conclusions should not be biased and should be based on the data, presented and discussed inside the manuscript only. Following the conclusion section, it is mandatory for manuscripts submitted for final publication in JUMJ to have a Gain of Knowledge summary that is consisted of 2 - 5 sentences that convey the core findings of the article.

Acknowledgements and Funding

Collate acknowledgements in a separate section at the end of the article before the references. List individuals or organizations that provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.). Whoever would be acknowledged should be informed and a verification for that could be requested by JUMJ Editor. If funded, the source of funding should be mentioned.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given

separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly, for tables and figures: Table A.1; Fig. A.1, etc.

CASE REPORT WRITING TEMPLATE

Title. Include the words “case report” in the title. Describe the phenomenon of greatest interest (e.g., symptom, diagnosis, diagnostic test, intervention, and outcome).

Abstract. Summarize the following information if relevant: 1) Rationale for this case report, 2) Presenting concerns (e.g., chief complaints or symptoms, diagnoses), 3) Interventions (e.g., diagnostic, preventive, prognostic, therapeutic exchange), 4) Outcomes, and 5) Main lesson(s) from this case report.

Key Words. Provide 3 - 8 key words that will help potential readers search for and find this case report.

Introduction. Briefly summarize the background and context of this case report.

Presenting Concerns. Describe the patient characteristics (e.g., relevant demographics - age, gender, ethnicity, occupation) and their presenting concern(s) with relevant details of related past interventions.

Clinical Findings. Describe: 1) the medical, family, and psychosocial history including lifestyle and genetic information; 2) pertinent co-morbidities and relevant interventions (e.g., self-care, other therapies); and 3) the physical examination (PE) focused on the pertinent findings including results from testing.

Timeline. Create a timeline that includes specific dates and times (table, figure, or graphic).

Diagnostic Focus and Assessment. Provide an assessment of the; 1) diagnostic methods (e.g., PE, laboratory testing, imaging, questionnaires, referral), 2) diagnostic challenges (e.g., financial, patient availability, cultural), 3) diagnostic reasoning including other diagnoses considered, and, 4) prognostic characteristics (e.g., staging) where applicable.

Therapeutic Focus and Assessment. Describe: 1) the type(s) of intervention (e.g., preventive, pharmacologic, surgical, lifestyle, self-care) and 2) the administration and intensity of the intervention (e.g., dosage, strength, duration, frequency).

Follow-up and Outcomes. Describe the clinical course of this case including all follow-up visits as well as 1) intervention modification, interruption, or discontinuation, and the reasons; 2) adherence to the intervention and how this was assessed; and 3) adverse effects or unanticipated events. In addition, describe:

1) patient-reported outcomes, 2) clinician-assessed and -reported outcomes, and 3) important positive and negative test results.

Discussion. Please describe: 1) the strengths and limitations of this case report including case management, 2) the literature relevant to this case report (the scientific and clinical context), 3) the rationale for your conclusions (e.g., potential causal links and generalizability), and 4) the main findings of this case report: What are the take-away messages?

Patient Perspective. The patient should share his or her experience or perspective of the care in a narrative that accompanies the case report whenever appropriate.

Informed Consent. Did the patient or their custodian give the author of this case report informed consent? Provide if requested.

Case Report Submission Requirements: 1) Competing interests, are there any competing interests? 2) Ethics Approval, Did an ethics committee or institutional review board review give approval? If yes, please provide if requested, 3) De-Identification, has all patient's related data been de-identified?

RANDOMIZED CLINICAL TRIALS WRITING TEMPLATE

In this particular type of original study, individuals are randomly allocated to receive or not receive a preventive, therapeutic, or diagnostic intervention and then followed up to determine the effect of the intervention. All randomized clinical trials should include a flow diagram and authors should provide a completed randomized trial checklist (see CONSORT Flow Diagram and Checklist; <http://www.consort-statement.org>) and a trial protocol.

Authors of randomized controlled trials are encouraged to submit trial protocols along with their manuscripts.

All clinical trials must be registered (before recruitment of the first participant) at an appropriate online public that must be independent of for-profit interest, e.g.:

- Research-SCDC@moh.gov.sa
- <https://sctr.sfda.gov.sa/>
- <http://www.clinicaltrials.gov>;
- <http://www.anzctr.org.au>;
- <http://www.umin.ac.jp/ctr>;
- <http://isrctn.org>;
- <http://www.trialregister.nl/trialreg/index.asp>.

Each manuscript should clearly state an objective or hypothesis; the design and methods (including the study setting and dates,

patients or participants with inclusion and exclusion criteria, or data sources, and how these were selected for the study); the essential features of any interventions; the main outcome measures; the main results of the study; a comment section placing the results in context with the published literature and addressing study limitations; and the conclusions.

Data included in research reports must be original. A structured abstract not exceeding 300 words is required. Clinical trials are limited to 2700 words (not including abstract, tables, figures, and references), 40 references, and no more than 5 tables and figures.

REVIEW, MINIREVIEW AND META-ANALYSIS PAPERS

These papers will not have empirical data acquired by the authors but will include historical perspectives, analysis and discussion of papers published and data acquired in a specific area.

Systematic reviews and meta-analyses are a particular type of original articles that perform systematic, critical assessment of literature and data sources pertaining to clinical topics, emphasizing factors such as cause, diagnosis, prognosis, therapy, or prevention. All articles or data sources should be searched for and selected systematically for inclusion and critically evaluated, and the search and selection process should be described in detail in the manuscript. The specific type of study or analysis, population, intervention, exposure, and tests or outcomes should be described for each article or data source. A structured abstract of less than 300 words is required. The text is limited to 3500 words (not including abstract, tables, figures, and references); about 4 tables (a flow diagram that depicts search and selection processes as well as evidence tables should be included) - and no reference limit.

Minireview is a brief historical perspective, or summaries of developments in fast-moving areas covered within the scope of the journal. They must be based on published articles; they are not outlets for unpublished data. They may address any subject within the scope of the journal. The goal of the minireview is to provide a concise very up-to-date summary of a particular field in a manner understandable to all readers.

SHORT COMMUNICATION AND SHORT RESEARCH ARTICLE

Short Communications are urgent communications of important preliminary results that are very original, of high interest and likely to have a significant impact on the

subject area of the journal. A Short Communication needs only to demonstrate a 'proof of principle'. Authors are encouraged to submit an Original Research Paper to the journal following their Short Communication. There is no strict page limit for a Short Communication; however, a length of 2500-3500 words, plus 2-3 figures and/or tables, and 15-20 key references is advisable. Short Research Article may be smaller single-result findings as a brief summary that include enough information, particularly in the methods and results sections, that a reader could understand what was done.

POLICY PAPER

The purpose of the policy paper is to provide a comprehensive and persuasive argument justifying the policy recommendations presented in the paper, and therefore to act as a decision-making tool and a call to action for the target audience.

COMMENTARIES/OPINION ARTICLES

An opinion-based article on a topical issue of broad interest, which is intended to engender discussion.

STUDY PROTOCOLS AND PRE-PROTOCOLS

JUMJ welcomes publishing protocols for any study design, including observational studies and systematic reviews. All protocols for randomized clinical trials must be registered and follow the CONSORT guidelines; ethical approval for the study must have been already granted. Study pre-protocols (i.e., discussing provisional study designs) may also be submitted and will be clearly labeled as such when published. Study protocols for pilot and feasibility studies may also be considered.

METHOD ARTICLES

These articles describe a new experimental or computational method, test or procedure, and should have been well tested. This includes new study methods, substantive modifications to existing methods or innovative applications of existing methods to new models or scientific questions. We also welcome new technical tools that facilitate the design or performance of experiments or operations and data analysis such as software and laboratory and surgical devices, or of new technologies to assist medical diagnosis and treatment such as drug delivery devices.

Maximum length of submissions

Full length original research articles should not exceed 10000 words (maximum 60 references), and up to 6 tables and/or figures.

Short communications comprising up to 1800 words of text, maximum 15 references, and two illustrative items (Tables and/or Figures).

Letters and Case Reports (provide novel insight into disease mechanisms, diagnostic and management applications). *Clinical Laboratory Notes* (technical evaluation or important insight into analytical methodology), or *Letters to the Editor* (focused on a specific article that has appeared in JUMJ within 4 weeks of print issue date of article). For all 3 types of letters listed above, the text should not exceed 600 words, with no abstract, a maximum of 1 table or figure and up to 5 references.

Review Articles, Surveys, Essays, and Special Reports may exceed the word and reference limit for Full-length articles as per the comprehensive nature of these articles. However, both of these articles (Reviews and Special Reports) will still require an abstract (unstructured, 350 word maximum).

Editorials, Meeting summary, Commentaries, Book review and Opinion pieces will not require an abstract and will be limited to 2000 words and up to 20 references. A book review is a brief critical and unbiased evaluation of a current book determined to be of interest to the journal audience. Publication of a submitted book review is at the discretion of the editor.

Artwork

General points

Make sure you use uniform lettering and sizing of your original artwork. Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier. Number the illustrations according to their sequence in the text. Use a logical naming convention for your artwork files. Indicate per figure if it is a single, 1.5 or 2-column fitting image. For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.

Formats

Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below). Please do not supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low, supply files that are too low in resolution, and, submit graphics that are disproportionately large for the content.

- EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.

- TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
- TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.
- TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures the Journal will ensure that these figures will appear in color on the Web regardless of whether or not these illustrations are reproduced in color in the printed version. Because of technical complications which can arise by converting color figures to 'gray scale' please submit in addition usable black and white versions of all the color illustrations.

Figure captions

Ensure that each illustration has a caption (Legend). A caption should comprise a brief title below the figure that describes its content and not to be general. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used in the legend. Figure caption should stand for itself (self-explanatory) without the need for consulting the text.

Tables

Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters within the table. If necessary, such footnotes could be placed at the end of the table title. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article (Figures or text). The table caption (Title) should be brief but describes its content and not to be general. Explain all symbols and abbreviations used in the table in the footnote. Table title should stand for itself (self-explanatory) without the need for consulting the text. The table structure should be scientifically organized (columns and rows) and its message should be easily comprehensible.

The Editor-in-Chief, on accepting a manuscript, may recommend that additional tables and/or graphs containing important backup data, too extensive to be published in the article, may be published as supplementary material. In that event, an appropriate statement will be added to the text. However,

the author should submit such material for consideration with the manuscript.

References

References cited should be relevant, up-to-date and adequately cover the field without ignoring any supportive or conflicting publications. Please ensure that every reference cited in the text is also present in the reference list (and vice versa). If present, unpublished results and personal communications may be mentioned in the text and not in the reference list. Citation of a reference as 'in press' implies that the item has been accepted for publication and shows up on PubMed literature search or a copy of the title page of the relevant article must be submitted. DOI of the references - whenever applicable should be presented. Authors are encouraged to cite primary literature rather than review articles in order to give credit to those who have done the original work.

Reference management software

This journal has standard templates available in key reference management packages EndNote

(<http://www.endnote.com/support/enstyles.asp>) and Reference Manager (<http://refman.com/support/rmstyles.asp>).

Using plug-ins to word processing packages, authors only need to select the appropriate journal template when preparing their article and the list of references and citations to these will be formatted according to the journal style, which is described below.

Reference formatting

There are no strict requirements on reference formatting at submission but should be consistent, complete and up-to-date. Where applicable, author(s) name(s), chapter title/article title, journal title/book title, year of publication, volume number-issue number/book chapter and the pagination must be present. For the book reference, the edition number, editors (if they are not the authors), publisher and its main address (City and Country) should be added as described below in the example. The reference style used by the journal should be applied to the accepted article at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. Use peer-reviewed references only except for national and international organizational reporting and registers. If you do wish to format the references yourself, they should be arranged according to the following examples:

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Indicate references by number(s) in curved brackets as a bolded superscript at the end of

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2. Arjmand MH, Ahmad Shah F, Saleh Moghadam M, Tara F, Jalili A, Mosavi Bazaz M, et al. Prooxidant-antioxidant balance in umbilical cord blood of infants with meconium stained of amniotic fluid. Biochem Res Int., 2013;2013:ID270545;1-4. DOI: 10.1155/2013/270545
3. Teferra RA, Grant BJ, Mindel JW, Siddiqi TA, Iftikhar IH, Ajaz F, Aliling JP, Khan MS, Hoffmann SP, Magalang UJ. Cost minimization using an artificial neural network sleep apnea prediction tool for sleep studies. Ann Am Thorac Soc., 2018 Jul 28 (ahead of print). DOI: 10.1513/AnnalsATS.201404-161OC
4. Alduraywish AA, Almani AZ, Alanazi AD-A, Alruwaili FS, Alolaywi AN, Almaeen AH, et al. Vitamin D insufficiency among healthy participants and type 2 diabetic patients from the northern Al-Jouf region of Saudi Arabia: Correlation with the prognostic indices of the disease. International Medical J, 2019 (Accepted for publication; <http://www.seronjihou.co.jp/imj/>).

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