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Edited by Prof. Gul Ozcan

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She is currently working as a distinguished professor in the Department of Biology at the Istanbul University. She received her M. Sc. and Ph.D degrees in Radiobiology and Health Physics, from the Institute of Graduate Studies in Science and Engineering at Istanbul University in 1995 and 2001, respectively. She worked at Istanbul University for about 29 years on molecular cancer biology and radiobiology. Presently, she is head of the molecular cancer biology and bioinformatics research laboratory. Over the past 10 years, her research has been focused on cell death mechanisms for cancer therapy, alternative death pathways, apoptosis resistance and cancer data mining. She has co- ordinate and contributed to several multi-disciplinary and multi-institutional research projects funded by the Scientific Research Projects Coordination Unit of Istanbul University. She is a fellow of the American Association for the Advancement of Science (AAAS), the European Association for Cancer Research (EACR), the Molecular Cancer Research Association (MOKAD) and the New York Academy of Sciences.

PREFACE

This book covers key areas of medical science. The contributions by the authors include high-grade gliomas, brain metastases, intracranial mass, solitary metastatic lesions, gingival fibromas, surface ulceration, diode laser, hip osteoarthritis, total hip arthroplasty, sickle cell disease, avascular necrosis, dental health, oral hygiene, pregnancy tumors, gingivitis, substance addiction, dna methylation pattern, drug addiction, environmental factor, soft tissue movement, orthognathic surgery, facial harmony, brachytherapy, cancer cervix, cancer management, time-tested low dose rate, radiation, eosinophilic oesophagitis, chronic oesophageal disease, dysphagia, chronic autoimmune reactions, liver organoid, pediatric diseases, organoid technology, 3D culture systems, apn/adipors signaling, choroidal neovascularization, inhibitory adiponectin peptide, eye ailments, management of ovarian masses, ovarian cancers, solid ovarian neoplasms, brenner's tumor, femoral fracture, pediatric orthopaedic injury, pediatric trauma, musculoskeletal injuries, oral squamous papilloma, human papilloma virus, papillary lesion, histopathological examination. This book contains various materials suitable for students, researchers, and academicians in the field of medical science.

Chapter 1
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Preoperative Soft Tissue Thickness Influences the Alteration of Soft Tissue Movement in Orthognathic Surgery

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ABSTRACT

Background: Reports on soft tissue movement after orthognathic surgery exhibit significant variability, with a lack of consensus on standardized measurements. This creates challenges in making accurate predictions about post-surgery tissue movement and determining the potential impact of preoperative soft tissue thickness on the extent of movement.
Aim: To investigate the relationship between preoperative soft tissue thickness and the degree of soft tissue movement in comparison to hard tissue movement following orthognathic surgery.
Materials and Methods: This study involves a retrospective analysis of lateral cephalometric X-rays obtained from patients who underwent orthognathic surgery at a single medical center between September 1, 2013, and September 1, 2018. Demographic and operative data were gathered. Preoperative cephalometric X-rays were used to measure soft tissue thickness, and postoperative X-rays (taken >6 months after surgery) were superimposed by aligning fixed bony points. Linear regression was employed to investigate the correlation between different variables and the extent of jaw movement.
Results: For upper jaw surgeries involving advancements up to 5 mm, there was an observed reduction in the relative movement of soft tissue, correlating with an increase in the initial thickness (r = -0.288). In mandibular advancements, there was a distinct decrease in the ratio of soft tissue movement with an increase in

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initial soft tissue thickness (r = -0.418). Conversely, there was no correlation in mandibular setback cases (r = 0.062). A similar correlation, demonstrating a decrease in relative soft tissue movement with an increase in initial soft tissue thickness, was identified in advancement genioplasty (r = -0.411). **Conclusion:** The findings of this research reveal a consistent pattern of decreased relative movement of soft tissue in orthognathic surgery, which is associated with an increase in its initial thickness.

Keywords: Orthognathic surgery; soft tissue movement; surgery prediction; surgery tracing.

1. INTRODUCTION

Orthognathic surgery, a procedure commonly employed to correct congenital and acquired facial deformities, focuses on jaw correction. This surgical intervention not only enhances the masticatory function of the dentition but also contributes to improved facial harmony and aesthetics. The sequence of events involves surgical movement of hard tissue, followed by subsequent adjustments in soft tissue. Consequently, various endeavors have been undertaken to explore and understand the correlation between changes in soft and hard tissue that occur post-orthognathic surgery [1–6].

Precise evaluation of both hard and soft tissue changes constitutes a crucial aspect of diagnosing and planning treatments in orthognathic surgery cases. This assessment plays a pivotal role in determining treatment success and offers patients a clearer understanding of the anticipated changes in their appearance following the surgery [7,8].

Earlier research has identified a correlation between the thickness of preoperative soft tissue and the subsequent changes that occur postoperatively due to movements in hard tissue [2,9–11]. The investigation revealed that the structure of facial soft tissue and the overall facial profile are reliant on the underlying bone structure. However, it was observed that changes in the bone do not manifest uniformly across all areas of the soft tissue [9,12,13]. As an illustration, research findings indicated that the movement of the upper lip corresponds to 60-90% of the movement observed in the underlying hard tissue, [8] while the movement of the lower lip aligns closely, ranging from 90-100% correlation with hard tissue movement, while in the chin, the correlation ranges from 55-90% [2,11,14].

Furthermore, there are reports asserting that the "relative connection" theory between soft and hard tissue is deemed inaccurate due to variations in soft tissue thickness [15]. The thickness of soft tissue plays a significant role in influencing the relative surgical disparities within it, emphasizing the necessity to take this factor into consideration when planning orthognathic surgery [16,17].

Soft tissue changes are assessed through a two-dimensional cephalometric evaluation conducted both before and after surgery. This evaluation can be carried out either manually or digitally, employing various digital software options,

sometimes in conjunction with video shots. Additionally, three-dimensional prediction methods are utilized in this assessment process [18].

Manual evaluation relies on anticipated changes, whereas digital software programs rely on average values from databases, as reported in previous studies that measured the connection between soft and hard tissue movement. Both methods provide limited and potentially inaccurate estimates of predicted soft tissue movement [19–21]. As a result, the precision of the simulations depends on the reliability and accuracy of the database utilized [1].

All manual techniques and the majority of digital methods assume that the soft tissue response is linear to the movement of hard tissue, irrespective of the pre-operative amount and movement direction of the skeletal tissue [1,8,19]. The validity of this assumption is not only subject to debate but also exhibits inconsistency in various reports from different research studies [15,22].

Additionally, previous reviews highlighted that due to differences in measuring and prediction methods, it was challenging to draw significant conclusions regarding soft tissue movement in this type of surgery [23].

The considerable variability and absence of a standardized measuring standard pose challenges in accurately assessing the outcomes of post-surgical soft tissue, as well as understanding the impact of pre-surgical soft tissue thickness on its movement ratio. The primary objective of this study was to investigate whether the thickness of preoperative soft tissue influences the extent of soft tissue movement in relation to hard tissue movement following orthognathic surgery.

2. MATERIALS AND METHODS

2.1 Definition of Study Sample

This study included all patients who underwent orthognathic surgery in the Department of Oral and Maxillofacial Surgery at the Baruch Padeh Tzafon Medical Center, Poriya, Israel, within the period from September 2013 to September 2018. Approval for the study was obtained from the Baruch Padeh Tzafon Institutional Review Board (IRB) under approval number POR-19-0109. A total of 42 patients who met the inclusion criteria, providing complete patient and surgery data, were enrolled in the study.

Exclusion criteria encompassed asymmetry cases requiring correction of differences in size or position of face halves, redo surgery cases, and cases with unavailable x-rays.

2.2 Definition of Variables

Dependent variable: Postoperative movement of soft tissue in millimeters (mm) in relation to operative hard tissue movement in mm [23a].

Independent variable: Preoperative thickness of soft tissue in mm.

2.3 Research Methods

The present study is a retrospective analysis conducted in accordance with the oral and maxillofacial surgery (OMFS) treatment protocol at the Baruch Padeh Tzafon Medical Center. As per the protocol, each orthognathic patient undergoes three lateral cephalometric X-rays: one within a month before surgery, another within the first 48 hours postoperatively, and a third six months postoperatively. All X-rays were consistently obtained using the same machine in the natural head position.

Measurements were carried out on these lateral cephalometric X-rays using on-screen digitization tools provided by Dolphin Ceph Tracing 11.95 software (Dolphin Imaging & Management Solutions, Chatsworth, CA, USA). The software allows for accurate distance measurements between two points on an image in millimeters following calibration with an "on image" ruler.

Data collected for each study participant included age, gender, medical condition, the operated jaw/area, and the type of operational movement (advancement or setback). Fig. 1 illustrates the measurements conducted for all patients, focusing on upper lip thickness (A-A'), lower lip thickness (B-B'), and chin soft tissue thickness (Pog-Pog').

The evaluation of hard and soft tissue operational changes involved superimposing preoperative and 6-month postoperative lateral cephalometric X-rays. This superimposition was accomplished by aligning constant hard tissue points unaffected by surgery, namely Nasion (N), Sella Turcica (S), and the S-N line.

Hard tissue changes were quantified using the following points:

- For patients undergoing maxillary surgery: A postop - A preop distance
- For patients undergoing mandibular surgery: B postop - B preop distance
- For patients undergoing chin surgery: Pog postop - Pog preop distance

Similarly, soft tissue changes were measured using the following points:

- A' postop - A' preop distance
- B' postop - B' preop distance
- Pog' postop - Pog' preop distance

Preoperative soft tissue thickness was determined based on measurements from the preoperative X-ray (A-A', B-B', Pog-Pog' – Fig. 1).

The relationship between postoperative hard and soft tissue movement was calculated by dividing the soft tissue movement by the corresponding hard tissue movement. $\frac{X'postop - X'preop}{Xpostop - Xpreop}$ (X=A/B/Pog).

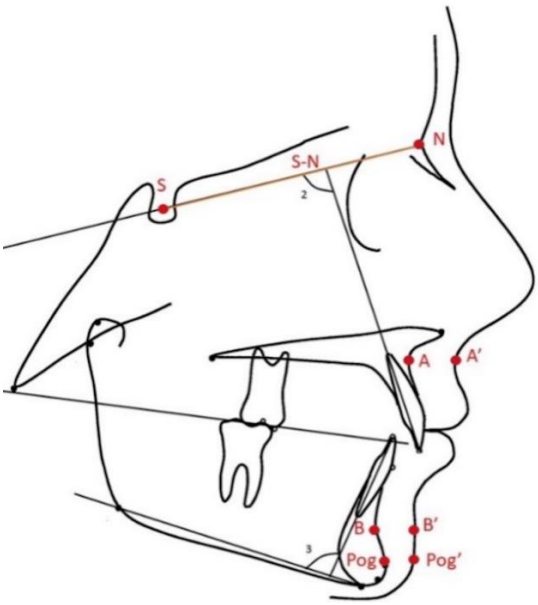


Fig. 1. Lateral cephalometric X-Ray measuring points. Abbreviations – S – Sella Turcica, N – Nasion, A – Hard tissue A point, A' – Soft tissue A point, B - Hard tissue B point, B' - Soft tissue B point, Pog - Hard tissue pogonion, Pog' - Soft tissue pogonion

2.4 Statistical Analysis

The statistical analysis for this study was conducted utilizing MS Excel (2021 version) and Prism GraphPad (9.0 version) software on MS Windows. Subgroup comparisons were carried out employing the t-test, Fisher's exact test, and the chi-square test. Adjustment factors were computed using the Pearson method. Linear regression was employed to assess correlations between variables and outcomes.

To compare correlations from independent samples, the Eid, Gollwitzer, and Schmidt method was employed. A significance level of <0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

This study comprised 42 patients with comprehensive available data, encompassing pre- and post-surgical imaging as well as detailed information about surgical movements. The mean age in this study was 21.8±6.9 years (range 16-32), with 27 female patients (64.3%) and 15 males (35.7%). Preoperative soft

tissue measurements are detailed in Table 1, while surgical hard tissue movement measurements are presented in Table 2 [23a].

Table 1. Preoperative soft tissue measurements

	N	Mean (± SD)	p value (t-test)
Preoperative soft tissue thickness upper lip (A-A' - mm)	33	13.79±2.37	
Preoperative soft tissue thickness upper lip - <5mm advancemnt (A-A' - mm)	16	14.06±2.77	0.53
Preoperative soft tissue thickness upper lip - >5mm advancemnt (A-A' - mm)	17	13.53±1.97	
Preoperative soft tissue thickness lower lip (B-B' - mm)	35	11.23±1.6	
Preoperative soft tissue thickness lower lip - setback cases (B-B' - mm)	21	11.75±1.46	**0.006**
Preoperative soft tissue thickness lower lip - advancement cases (B-B' - mm)	14	10.28±1.46	
Preoperative soft tissue thickness upper lip (Pog-Pog' - mm)	6	11.8±2.44	

Table 2. Surgical hard tissue movement measurements

	Mean (±SD)
Maxillary AP advancement (N=33, mm)	5.55±2.12
Mandibular AP advancement (N=14 mm)	5.96±2.42
Mandibular AP setback (N=21, mm)	-6.21±2.52
Chin AP advancement (N=6, mm)	6.02±3.2

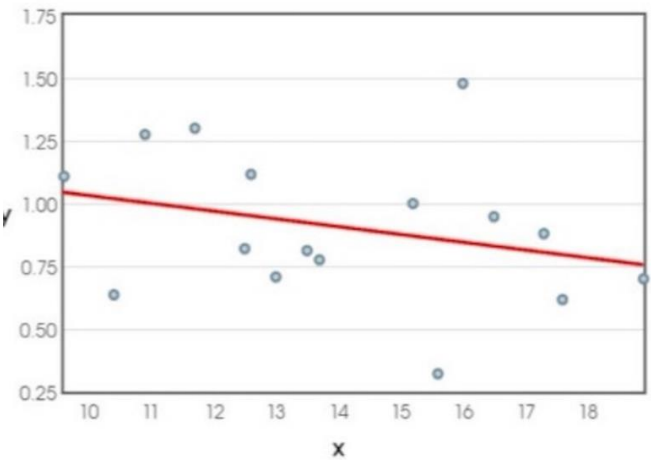
Bi-jaw surgery was the most prevalent type, accounting for 57.1% of cases, followed by mandible-only surgery in 16.7% of cases (Table 3) [23a]. Among the cases involving maxillary advancement (33 cases), the mean preoperative soft tissue thickness (A-A') was 13.79±2.37mm (range 9-18.9). The mean surgical hard tissue advancement was 5.55±2.12mm (range 1.6-9.1). Linear regression analysis of the correlation between preoperative soft tissue thickness and the relationship between hard and soft tissue postoperative movement in maxillary advancement cases did not reveal a significant correlation (r=-0.101, p=0.58). However, a general trend indicated a decrease in relative soft tissue movement with an increase in its initial thickness.

Further analysis within cases of less than 5mm maxillary advancement (16 cases) demonstrated a clear, albeit not statistically significant, trend of decreased relative movement of postoperative soft tissue with an increase in its initial thickness (r=-0.288, p=0.28, Fig. 2) [23a]. In cases of >5mm maxillary advancement (17 cases),

there was a trend, although not statistically significant, of an increase in relative soft tissue movement with an increase in its preoperative thickness (r=0.308, p=0.22, Fig. 3) [23a]. No statistically significant difference in pre-surgical soft tissue thickness was observed between the two groups (p=0.53, Table 1).

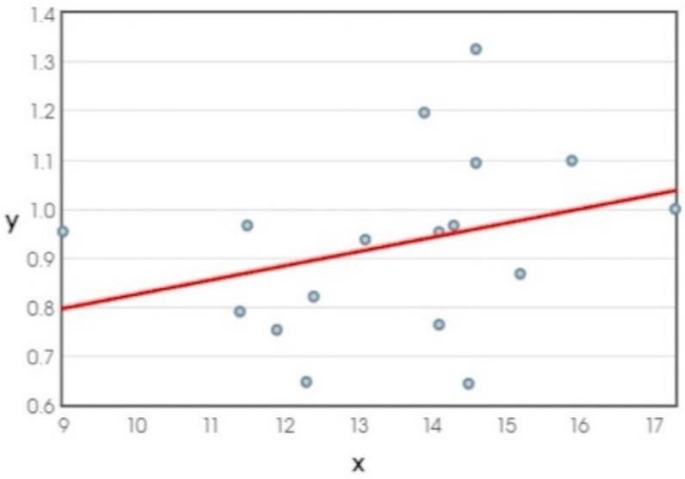
Table 3. Prevalence of different surgery types

Type	Count	%
Bi-Maxillary (Two Jaws)	24	57.1%
Bi-Maxillary and chin	4	9.5%
Maxilla only	5	11.9%
Maxilla and chin	1	2.4%
Mandible only	7	16.7%
Mandible and chin	1	2.4%
Total	**42**	**100.0%**



Regression Line: $y = -0.031x + 1.3419$
Correlation: $r = -0.2882$
R-squared: $r^2 = 0.0831$

Fig. 2. Correlation between initial soft tissue thickness in the maxilla (x axis) and the relation between postoperative soft and hard tissue movement (y axis) – cases of maxillary advancement of 5mm or less (n=16, p=0.28)



Regression Line: $y = 0.029x + 0.5352$
Correlation: $r = 0.3082$
R-squared: $r^2 = 0.095$

Fig. 3. Correlation between initial soft tissue thickness in the maxilla (x axis) and the relation between postoperative soft and hard tissue movement (y axis) – maxillary advancement of more than 5 mm (n=17, p=0.23)

Among cases involving horizontal movement of the mandible (35 cases), the mean preoperative soft tissue thickness (B-B') was 11.23±1.6mm. Linear regression analysis of the correlation between preoperative soft tissue thickness and the relationship between hard and soft tissue postoperative movement in mandibular horizontal movement cases showed no correlation or trend (r=-0.011, p=0.95). Stratifying the analysis into advancement and setback movements separately revealed a statistically significant difference in pre-surgical tissue thickness between the groups (p=0.006), with the setback group having initially thicker soft tissue. In setback cases (21 cases – mean surgical hard tissue movement -6.21±2.52mm), there was no significant trend (r=0.06, p=0.79, Fig. 4) [23a]. However, in mandibular advancement (14 cases – mean surgical hard tissue movement 5.96±2.42mm), there was a clear and borderline significant trend of decreased relative soft tissue movement with an increase in its preoperative thickness (r=-0.417, p=0.12, Fig. 5) [23a].

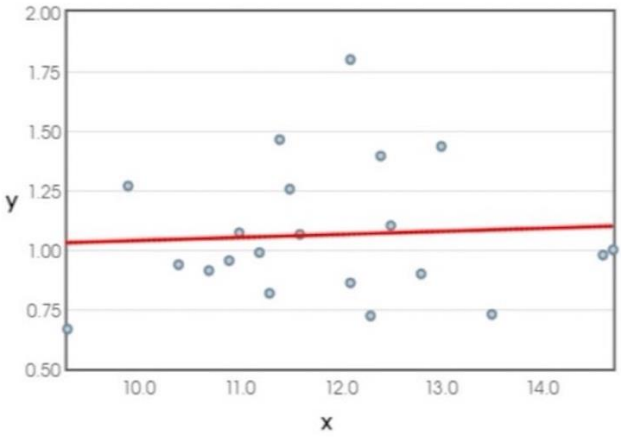
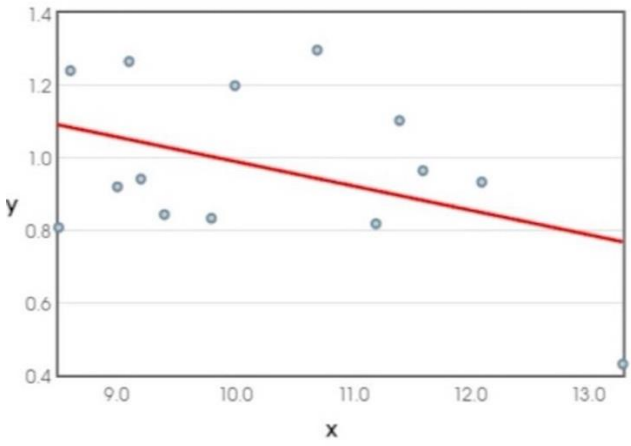


Fig. 4. Correlation between initial soft tissue thickness in the mandible (x axis) and the relation between postoperative soft and hard tissue movement (y axis) – mandibular setback cases (n=21, p=0.79)

In cases involving horizontal advancement of the chin (six cases), the mean preoperative soft tissue thickness (Pog-Pog') was 11.8±2.44mm. The mean surgical hard tissue advancement was 6.02±3.2mm. Linear regression analysis of the correlation between preoperative soft tissue thickness and the relationship between hard and soft tissue postoperative movement in chin advancement cases showed a clear, albeit not statistically significant, trend of decreased relative soft tissue movement with an increase in its preoperative thickness (r=-0.411, p=0.42).

3.2 Discussion

In this retrospective research, cephalometric X-rays of orthognathic surgery patients were analyzed to assess the preoperative thickness of their soft tissue and the extent of its movement in various types of surgeries involving the maxilla, mandible, and chin. The primary objective was to investigate whether preoperative soft tissue thickness influences the relative degree of soft tissue movement in orthognathic surgery.



Regression Line: $y = -0.0673x + 1.6638$
Correlation: $r = -0.4178$
R-squared: $r^2 = 0.1746$

Fig. 5. Correlation between initial soft tissue thickness in the mandible (x axis) and the relation between postoperative soft and hard tissue movement (y axis) – mandibular advancement cases (n=14, p=0.12)

The analysis was categorized into three groups: surgeries involving the maxilla, surgeries involving the mandible, and surgeries involving the chin. For maxillary surgeries (33 cases), an overall trend indicated a decrease in relative soft tissue movement with an increase in its initial thickness (r=-0.101). However, when cases were further divided into those involving 5mm or less advancement (16 cases) and those with more than 5mm advancement (17 cases), a distinct pattern emerged. In cases with ≤5mm advancement, there was a clear trend of decreased relative soft tissue movement with an increase in its initial thickness (r=-0.2882). Conversely, in cases with >5mm advancement, the trend was opposite, showing an increase in relative soft tissue movement with an increase in its preoperative thickness (r=0.3082).

This stratification aligns with previous reports, indicating that in cases of >5mm maxillary advancement, predicting soft tissue relative movement becomes more challenging [24–26].

One plausible explanation for the observed decrease in relative soft tissue movement with an increase in its initial thickness could be linked to the fact that thicker soft tissue might absorb a portion of the operative changes to the hard

tissue. In cases where the soft tissue is thicker, it may act as a cushion, mitigating the impact of surgical alterations on the overall movement of the soft tissue. This absorption effect could contribute to the trend of reduced relative soft tissue movement in such instances [24]. Furthermore, multiple previous reports have consistently demonstrated that a higher initial soft tissue thickness is associated with relatively smaller movement, correlating with changes in hard tissue. This recurrent finding supports the notion that the thickness of the initial soft tissue plays a role in influencing the extent of its movement in relation to changes in hard tissue [10,24,27].

The unexpected finding in cases of >5mm maxillary advancements, showing a clear trend of an increase in relative soft tissue movement with an increase in its initial thickness, may be attributed to the limitation of soft tissue, even when thick, to effectively absorb relatively large movements (>5mm). Previous reports in analogous movements have consistently highlighted the considerable challenge in predicting relative soft tissue movement and achieving a robust correlation, particularly in cases involving substantial movements. This underscores the complexity of the interaction between soft and hard tissue dynamics in orthognathic surgeries, especially when dealing with larger adjustments [10,12,25].

In surgeries involving the mandible (35 cases), it was challenging to identify a clear general trend due to the significant differences between the characteristics of advancement and setback surgeries. However, when setbacks (21 cases) and advancements (14 cases) were analyzed separately, a distinct pattern emerged. In advancement cases, there was a clear trend of decreased relative soft tissue movement with an increase in its initial thickness ($r=-0.4178$), whereas setback cases did not exhibit any clear trend ($r=-0.0625$). Additionally, setback cases demonstrated significantly thicker pre-operative soft tissue compared to advancement cases. This suggests that the relationship between initial soft tissue thickness and subsequent movement varies depending on the nature of the mandibular surgical procedure.

The observed trend of a decrease in relative soft tissue movement with an increase in its initial thickness in mandibular advancement cases may be explained similarly to the phenomenon observed in small advancements of the maxilla. The absorption of hard tissue movement by thick soft tissue could account for this pattern, indicating a potential cushioning effect in cases where the soft tissue is initially thicker. This explanation aligns with the concept that greater soft tissue thickness may serve as a buffer against the impact of surgical changes to the hard tissue, influencing the overall movement dynamics [24]. In setback cases involving the mandible, it is not feasible to identify any discernible trend, which is consistent with findings from previous reports. This aligns with the observation of very low regression coefficients for the correlation between hard and soft tissue movement in mandibular setback cases. The complexity and variability associated with mandibular setback surgeries may contribute to the difficulty in establishing a clear trend in the relationship between initial soft tissue thickness and subsequent movement in these cases [6,20,28,29]. Same findings were presented in a

systematic review on this subject [30]. The primary explanation for the observed differences in trend between advancement and setback surgeries in mandibular procedures is closely linked to the nature of the surgical movement itself. In advancement surgeries, there is an anterior pulling of the tissue, leading to stretching, and the extent of this stretch is contingent upon the tissue's ability to absorb subsequent tension—a quality largely dictated by its initial thickness. Conversely, in setback surgery, the soft tissue doesn't move posteriorly in perfect alignment with the hard tissue, diminishing the clear influence of initial soft tissue thickness. This distinction is accentuated by the significant difference in initial tissue thickness between the groups, with setback patients having, on average, 1.5 mm more pre-surgical soft tissue thickness than advancement patients.

In chin advancement surgeries (six cases), a clear trend of decreased relative soft tissue movement with an increase in its initial thickness was observed (r=-0.4113). Once again, this trend may be attributed to the absorption of hard tissue movement by thick soft tissue, as noted in this study and supported by previous reports [24].

The limitations of this research include:

1. Multiple Jaw Surgeries: Some of the conducted surgeries involved more than one jaw. This introduces a situation where similar movements are examined in different surgeries, potentially influencing the outcomes as the soft tissue of one jaw may be affected by the surgery of the other. Previous studies have suggested a distinction in soft tissue mobility between single-jaw and bimaxillary surgeries [31,32].
2. Small Study Group: The study group was relatively small, and cases with insufficient data were excluded, posing challenges in achieving statistically significant results.
3. Limitations in Cephalometric X-ray Analysis: The ability to assess the relationship between hard and soft tissue movements in lateral cephalometric X-rays is constrained by various factors such as complex anatomical structures, image superimposition, image sharpness and quality, and the researcher's precision in marking reference points.

Considering these limitations, future research efforts could benefit from multi-center, multi-national collaborations with a larger and more diverse patient pool. Such studies should aim to isolate different types of surgeries, allowing for more robust statistical analyses of each movement within each type of surgery.

4. CONCLUSION

In this study, a general trend was observed indicating a decrease in the relative surgical movement of soft tissue with an increase in its initial width. The only exception was noted in cases of >5mm maxillary advancement, where results were contrary, potentially influenced by the substantial movement involved in the surgery itself. Further research with larger study samples, preferably prospective studies, is warranted to obtain clearer and more significant results. Analyzing this data could contribute to improving the accuracy of pre-surgical planning and

predicting outcomes in orthognathic surgeries. This, in turn, could lead to enhanced surgical outcomes, better alignment with patients' needs, and improved anticipation of their expectations.

ETHICAL APPROVAL

All authors hereby declare that the research was approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 2
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Characterization of High-Grade Glioma and Solitary Metastasis: An Approach towards Spectroscopy and Advanced Magnetic Resonance Techniques

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ABSTRACT

Aim: The purpose of this article is to analyze different advanced MRI parameters and characterize the efficacy of each one of them in the differentiation of these two pathologies.
Background: The differentiation by means of magnetic resonance between high-grade gliomas and intracranial solitary single metastasis is of the utmost importance since they condition both surgical and complementary treatment. For decades, different methods have been devised to try to differentiate both, given that the final result of surgery and complementary treatment differs substantially between the two. What's more, knowing the possible diagnosis in advance would improve the prognosis of both pathologies.
Methods: Retrospective study that analyzes the parameters of advanced magnetic resonance imaging: spectroscopy, diffusion and perfusion, specifically focused on the differences in the coefficients of the metabolites Cho/Cr, Cho/ NAA and NAA/Cr in peritumoral edema between high-grade gliomas and metastases. The data have been statistically analyzed using ROC (receiver operating characteristic) curves, and cutoff values were obtained.
Results: A total of 79 patients with histologically analyzed tumors were analyzed: 49 high-grade gliomas (40 multiform glioblastomas and 9 anaplastic astrocytomas) and 30 metastases. A statistically significant mean difference was obtained in the three metabolite ratios. The area under the curve for the Cho/NAA ratio was 0.958

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(CI: 0.903–1), for Cho/Cr 0.922 (CI: 0.859–0.985) and for NAA/Cr 0.163 (CI: 0.068–0.258; $p < 0.001$). The cutoff values were 1.115 for Cho/NAA (sensitivity 93.87%, specificity 93.33%, global precision 93.67%); 1.18 for the Cho/Cr ratio (sensitivity 89.79%, specificity 93.33% and precision 91.13%) and 1.155 for the NAA/Cr ratio (sensitivity 67.34%, specificity 93.33%, global precision 44.30%).

Conclusion: The results of the study support the premise that spectroscopy at the level of peritumoral edema is able to differentiate between high-grade gliomas and metastases by showing tumor infiltration in peritumoral edema. This difference allows not only tumor diagnosis but also the possibility of performing high-grade glioma surgery guided by spectroscopy to find tumor infiltration in apparently healthy tissue.

Keywords: Metastasis; high-grade gliomas; magnetic resonance; spectroscopy; glioblastoma multiforme.

ABBREVIATIONS

AA : Anaplastic Astrocytomas
AUC : Area Under the Curve
Cho : Choline
CI : Confidence Interval
Cr : Creatinine
CV : Cutoff Value
E : Specificity
GBM : Glioblastoma Multiforme
LL : Lipids and Lactate
MI : Myoinositol
MRI : Magnetic Resonance Imaging
MRS : Magnetic Resonance Spectroscopy
NAA : N-Acety-Laspartate
NPV : Negative Predictive Value
PPV : Positive Predictive Value
rCBV : Relative Cerebral Blood Volume
ROC : Receiver Operating Characteristic
ROI : Region of Interest
S : Sensitivity
TE : Echo Time
TR : Repetition Time

1. BACKGROUND

High-grade gliomas and solitary brain metastases have similar imaging appearances, which often leads to misclassification [1]. Brain metastases are the most common intracranial tumors, affecting 20–40% of cancer patients. Its main form of presentation is as a single intracranial mass. In this same way, high-grade primary brain tumors (glioblastoma multiforme and anaplastic astrocytomas) often present [1a,2,3]. Given the clear difference between the medical–surgical management and the survival of these two pathologies, its diagnosis prior to

surgery is of vital importance. Even the surgery itself can be altered by the tumor diagnosis since the metastasis has a clear dissection plane that allows its complete excision more easily. High-grade glioma is a malignant tumor characterized by infiltration of the adjacent parenchyma.

It is considered a systematic disease per se although its ability to metastasize is lower than what would be expected due to its degree of malignancy.

Magnetic resonance imaging (MRI) is the fundamental diagnostic tool available to the neurosurgeon for this purpose.

For many years, conventional techniques have had limitations in this regard, given the similarities in the presentation of these two pathologies [1a]. With the arrival of advanced techniques (spectroscopy, perfusion and diffusion), anatomical information is combined with physiological and metabolic information, and in this way, the differentiation between the two begins to glimpse a way out [4].

The purpose of this article is to analyze different advanced MRI parameters and characterize the efficacy of each one of them in the differentiation of these two pathologies.

2. METHODS

A retrospective analysis was performed about brain tumor cases operated at the Neurosurgery Service of the Hospital Universitario de la Princesa from 2016 to 2018 [1a].

The selection criteria were the presence of a single intracranial tumor lesion and the performance of advanced pre-surgical imaging techniques. The presence of spectroscopy was considered mandatory, the presence of diffusion or perfusion being optional. Patients with a previous history of surgery, radiotherapy or chemotherapy or with more than one lesion were excluded.

A total selection of 79 patients was obtained. Advance MRI techniques were performed prior patients consent. The data are synthesized in Table 1 [1a].

2.1 MRI Imaging Technique

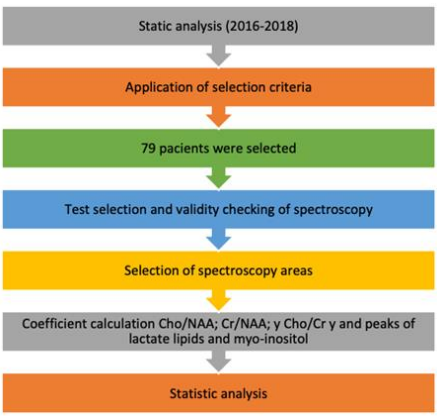
The image was taken with a Siemens 1.5 T system (Magnetom Sonata; Siemens, Erlangen, Germany). The MRI imaging protocol included T2 - and T1 -weighted pre-contrast transverse echo-spin images. In each study, T2-weighted images were obtained using a rotational echo sequence with a TR/TE of 4000/100 and a slice thickness of 3 mm. T1-weighted pre-contrast images were acquired with a slice thickness of 3 mm and a TR/TE of 800/9 [1a].

2.2 Spectroscopy

Spectroscopic data were obtained after administration of gadopentetate dimeglumine. Three-dimensional selective excitation was used to excite a rectangular section, and the size of the section depended on the location and size of the lesion [1a]. The section was positioned in the peritumoral region and the

normal contralateral brain parenchyma as a control, avoiding contamination of the scalp fat. The peritumoral region was defined within 1 cm outside the external tumor margin [5].

Table 1. Diagram of the work process



Spectral maps were obtained with an 8 × 8 matrix, having a voxel size of 1 × 1x1.5 cm, or 1 × 1x2 cm, depending on the thickness of the section. The term "spectroscopic MR imaging" is used synonymously with multivoxel spectroscopy, MR spectroscopic imaging or chemical shift imaging spectroscopy. All spectroscopic MR studies were multivoxel and/or chemical shift acquisitions and/or MR spectroscopy imaging [1a].

2.3 Data Processing

Spectroscopic data were processed using an offline workstation with standard software (Siemens) [1a]. The spectral data were automatically corrected, except for the cases that seemed distorted in which the manual method was used.

The Gaussian filters were automatically adjusted to the peaks of choline (Cho), creatinine (Cr) and N-acetylaspartate (NAA), and the following coefficients were calculated: Cho/NAA; Cr/NAA; and Cho/Cr. The lipid and lactate (LL) peaks were also obtained in addition to the possible myoinositol (MI) peaks.

The metabolic relationships in the multiple voxels were calculated; however, only the maximum values in three locations were included, depending on the metabolite to be analyzed (intratumoral, the peritumoral region and in the normal contralateral brain parenchyma), and the spectra with these maximum values were identified from spectral maps [1a].

Metabolite values were automatically calculated from the area under each metabolite peak using the standard commercial software program provided by the manufacturer. The integral peak values were normalized to the internal Cr peak.

2.4 Statistic Analysis

The metabolic ratios obtained from spectroscopic MRI data between high-grade gliomas and metastases were compared using Student's t test. A P value less than 0.05 indicated a statistically significant difference [1a].

The comparison between the different proportions in the expression of different metabolites such as LL, general decrease in metabolites or increase in myoinositol was calculated using Fisher's exact test. This test has a significant level of 5%. An analysis of the results was also carried out using the ROC curve (reception operating curve). Analyses based on logistic regression models were used to find the optimal cutoff value (sensitivity/ specificity ratio) for metabolite ratios in order to achieve the most accurate diagnostic prediction possible [1a]. The area under the curve or AUC was taken as a criterion. AUC values of 51–70%, 71–90%, and > 90% indicate low, moderate and high diagnostic accuracy, respectively. Sensitivity (S), specificity (E), positive predictive value (PPV), negative predictive value (NPV) and precision for optimal thresholds were subsequently detailed. Precision was defined as the number of truly identified patients divided by the total number of cases. The Statistical Pack-age for the Social Sciences software (Version 15.0; SPSS, Chicago, Illinois) was used for the analysis of statistical data. Optimal thresholds were subsequently detailed. Precision was defined as the number of truly identified patients divided by the total number of cases. The Statistical Pack-age for the Social Sciences software (Version 15.0; SPSS, Chicago, Illinois) was used for the analysis of statistical data.

Table 2. Description of the initial symptoms of the study patients in which it can be seen that the epileptic seizure is the most frequent symptom

Patients symptoms	Frequency	Percentage (%)
Epileptic seizures	28	35.4
Headache/vomiting/nausea	10	12.7
Paresthesia/hemiparesis	15	19
Language impairment	7	8.9
Vision impairment	1	13
Gait disturbance	6	7.6
Behavior alteration	5	6.3
Disorientation	2	2.5
Casual	1	1.3
General impairment	3	3.8
Others	1	1.3

3. RESULTS

3.1 Descriptive Analysis

The total selection consists of 79 patients (43 men, 36 women; ages between 30 and 85 years with a mean of 60.96 years). The predominant debut symptom was the seizure present in 28 patients, representing 35.4.9%

of the total, and the second most frequent was the onset with paresthesia or hemiparesis present in 15 patients (19%). You can see the distribution in Table 2 [1a].

The lesions were distributed in different locations, pre-dominantly the frontal lesion in 30 patients (38%) and the pure temporal lesion in 17 patients (24.1%).

The lesions were distributed in practically the same proportion between the right and left hemispheres (48.1% and 46.8%). Four patients presented central location with bilateral extension (5.1%) [1a].

Spectroscopy studies were performed in 79 patients, perfusion studies were performed in 79 patients, diffusion studies were performed in 76 patients, and anisotropy studies were performed in 72.

In a total of 69 patients, a complete study was per-formed using spectroscopy, diffusion, perfusion and anisotropy: 39 patients with primary tumors and 30 patients with metastases. The radiologist was responsible for the choice of advanced MRI techniques [1a].

The pathological examination revealed that 49 patients histologically presented a high-grade tumor (glioblastoma multiforme) and anaplastic forms (AA) according to the classification of the World Health Organization. Of the 49 patients with high-grade tumors, 41 corresponded to glioblastoma multiforme and 8 to anaplastic astrocytoma. Of the 30 patients with metastases, 2 were derived from gastrointestinal tumors, 6 from breast cancer, 1 from the ovary, 2 from melanoma, 2 from the pancreas and 16 from the lung (adenocarcinoma, neuroendocrine and small cells). Seventy-two open surgeries (88.3%) and 7 biopsies (11.7%) were performed. Results are shown in Fig. 1 [1a].

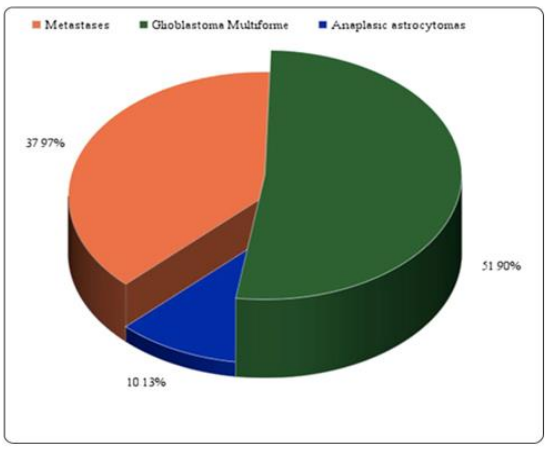


Fig. 1. Description of the percentage of the different tumor types in the study

3.2 Statistic Analysis

A comparison was made between coefficients of metabolites found in peritumoral edema.

The mean of the Cho/Cr coefficient in high-rank and metastatic gliomas was: 1.5931 ± 0.4248 and 0.9201 ± 0.1964, whose difference was statistically significant ($p < 0.001$).

The mean of the Cho/NAA coefficient between high-ranking gliomas and metastases was: 1.4845 ± 0.40881 and 0.7697 ± 0.2302, whose differences were statistically significant ($p < 0,001$).

The mean of the NAA/Cr coefficient among high-ranking and metastatic gliomas was: 1.1857 ± 0.089 and 1.289 ± 0.0839, whose differences were not significant (p: 0.05).

The data are synthesized in Table 3 [1a]. By making the disaggregated comparisons between GBM/metastasis and AA/metastasis, significant indices were also obtained at the level of peritumoral edema except in the NAA/Cr coefficient. The results can be viewed in Table 4 [1a].

The comparison made between AA and GBM did not yield any significant data.

Lipids and lactates: Intratumor expression of lipids (at 0–9 and 1–3 ppm) and lactate (1.35 ppm) was detected both at the level of high-grade gliomas and metastases. The proportion within the GBM/AA group was 44 cases with a positive peak (GBM: 40, AA: 4) and 5 with a negative peak. In the metastasis group, the relationship was 27 positives versus 3 negatives [1a]. No statistically significant differences were found when the chi-square test was per-formed, so this metabolite does not serve to differentiate both pathologies.

3.3 Other Variables

Regarding the generalized decrease in metabolites, it was found that 14 of 48 high-grade gliomas presented it. Regarding metastases, they were 6 out of 30 [1.a]. These data are not significant and do not show a characteristic pro-file of either of these two pathologies. The analysis was also not significant when performed by subgroups.

Table 3. Results of the MRS coefficients in peritumoral edema in high-grade gliomas and metastases

Tumor group	Cho/Cr mean (± SD)	Cho/NAA mean (± SD)	NAA/Cr mean (± SD)
High-grade gliomas (*n*:49)	1.59 ± 0.42	1.48 ± 0.41	1.19 ± 0.09
GBM (*n*:40)	1.57 ± 0.43	1.53 ± 0.42	1.18 ± 0.70
AA (*n*:8)	1.68 ± 0.41	1.28 ± 0,30	1.22 ± 0.15
Metastases	0.92 ± 0.19	0.77 ± 0.23	1.29 ± 0.08

SD: Standard Deviation

Table 4. Comparison of MRS coefficients in peritumoral edema for high-grade gliomas, GBM, AA and metastases

Comparison	Cho/Cr	Cho/NAA	NAA/Cr
High-grade gliomas Versus metastases	*P* < 0.0001	*P* < 0.0001	*P*: 0.05
GBM versus metastases	*P* < 0.0001	*P* < 0.0001	*P*: 0.209
AA versus metastases	*P*: 0.005	*P*: 0.004	*P*: 1.49

There was a slight statistically positive difference (p: 0.049) between the expression of a myoinositol peak between the AA group and the GBM group.

3.4 ROC Curves

By making a comparison between the three metabolite coefficients, we can deduce that Cho/NAA has greater discriminative power than the other tests in the identification of high-grade gliomas versus metastases: Area under the curve (confidence interval) 0.958 (CI: 0,903–1 vs. Cho/Cr 0.922 (CI: 0.859–0.985) and NAA/Cr 0.163 (CI: 0.068–0.258; p < 0.001 (Fig. 2) [1a].

The NAA/Cr coefficient presents an area under the curve of 0.837 (0.742–0.932) in the identification of metastases against high-grade gliomas (Fig. 3) [1a].

3.5 Optimal Cutoff Values

ROC analysis for the differentiation between metastasis and high-grade gliomas yielded an optimal cutoff value of 1.115 for the peritumoral Cho/NAA ratio variable [1a]. This implies a proportion of correctly identified high-grade gliomas (sensitivity) of 93.87%, a proportion of correctly identified metastases (specificity) of 93.33%, the proportion of true high-grade gliomas identified as such (PPV) was 95.83% and the proportion of true metastases identified as such (NPV) was 95.83%. The overall test precision was 93.67%

The cutoff value for other variables was> or equal to 1.18 for the Cho/Cr coefficient (sensitivity of 89.79%, specificity of 93.33%, positive predictive value of 95.65%

and negative predictive value of 84.84% and a precision of 91.13%) and 1.155 for the NAA/Cr coefficient (sensitivity of 67.34%, specificity of 93.33%, PPV of 54.09%, NPV of 11.11% and global precision of 44.30%). For the Cho/Cr and Cho/NAA variables, a high value of the coefficient corresponds to high -grade gliomas and a low grade to metastasis. On the contrary, a high value of the NAA/Cr coefficient corresponds to metastasis (Fig. 4) [1a].

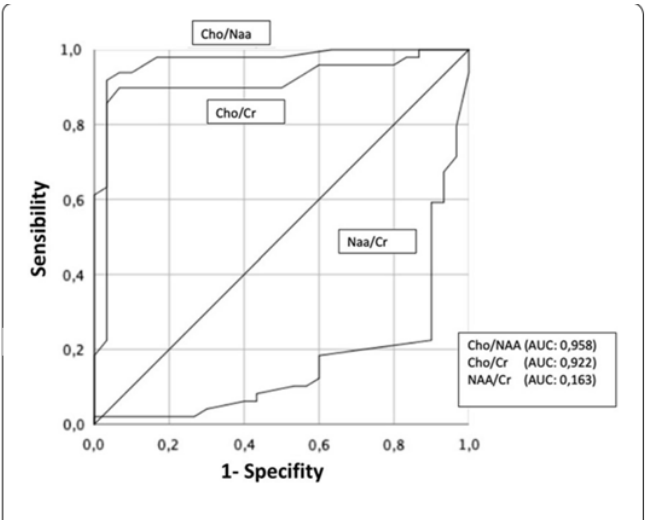


Fig. 2. Graph showing a ROC (receiving operating characteristic) curve of the peritumoral variables Cho/NAA, Cho/Cr and NAA/Cr for the differentiation of high-grade gliomas and metastases. This graph shows the discriminative capacity of each variable, also providing the areas under the curve (AUC)

The details of the ROC curves are shown in Table 5 [1a].

3.6 Perfusion

A qualitative analysis was performed. An increase in global arterial flow was found in 45/49 high-grade gliomas and in 17/30 metastases. The difference was significant p: 0.002 [1a].

The most frequent found pattern was peripheric increase and central decrease in arterial flow. This pattern was expressed in 17/49 high-grade gliomas and in only 1 metastasis. This difference was statistically significant.

3.7 Anisotropy

A qualitative analysis was performed. The anisotropy study was present in 72 of 79 patients. Fifty-four patients had a loss of local fractional anisotropy or brain

microstructure. When comparing groups, a statistically significant difference was found with a trend of greater local aggressivity for high-grade glioma group.

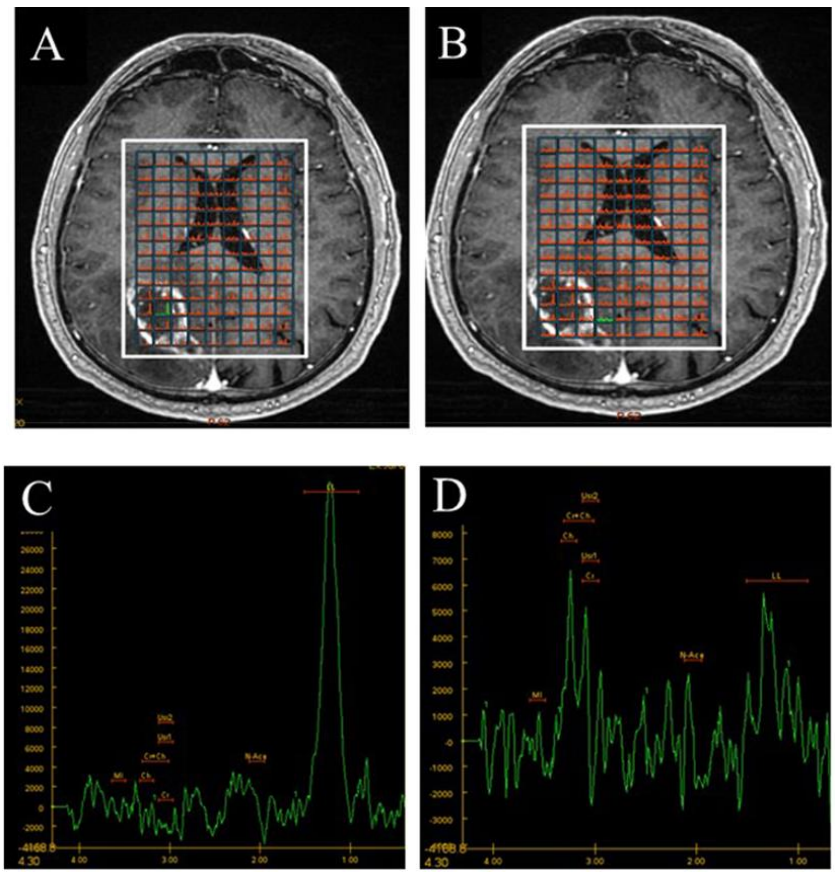


Fig. 3. A 68-year-old male patient with a brain tumor in the right parietal region confirmed histologically as a grade IV astrocytic tumor (astrocytoma). A and B axial MR image in T1 sequence with contrast. A heterogeneous lesion with irregular ring-shaped enhancement and central necrosis can be seen. Perilesional edema is noted in the form of hypodensity in T1 sequence (vasogenic edema and tumor infiltration); C spectroscopy of the tumor area with elevated lipids and lactates in addition to a generalized decrease in the rest of the metabolites; typical of areas with necrosis and ischemia; D: spectroscopy of the peritumoral area with a characteristic pattern of increased Cho and Cr together with decreased NAA

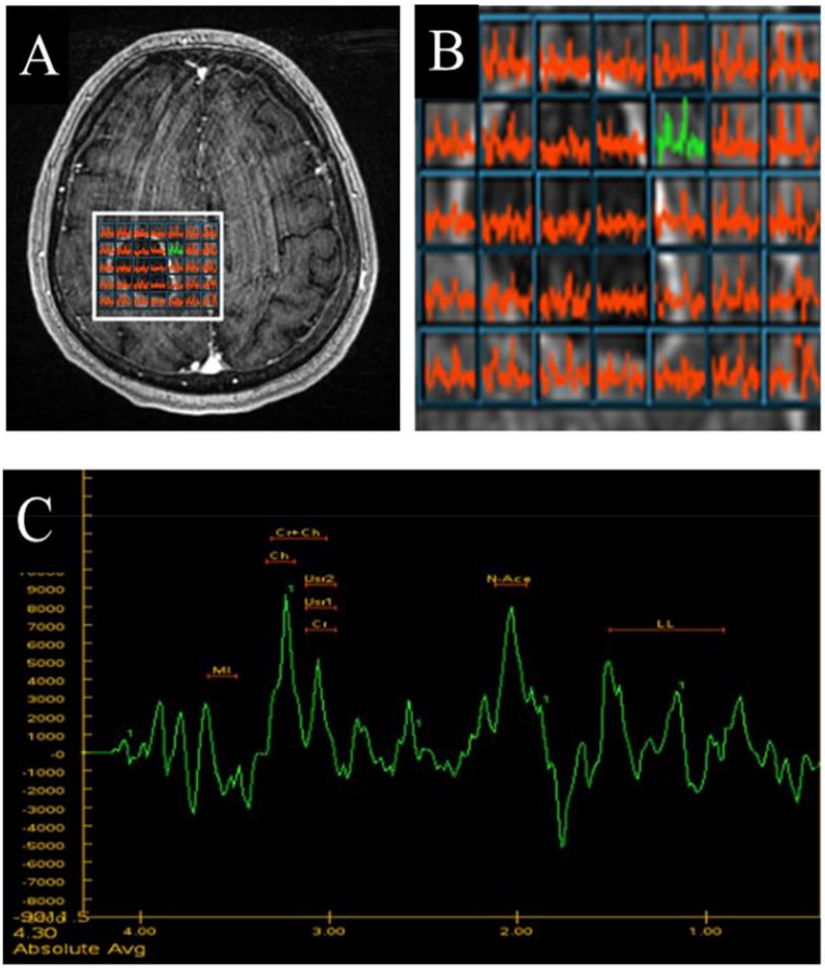


Fig. 4. A 57-year-old male patient with a histologically confirmed right prerolandic frontal lesion as metastasis from lung cancer. A Axial T1-weighted MR image with contrast. An irregular linear peripheral enhancement lesion with thickenings is visualized; B spectroscopic map in which the lack of choline elevation in the peritumoral voxels can be confirmed; C spectroscopic analysis showing a Cho peak and a not so pronounced NAA drop

Table 5. Measures of sensitivity, specificity, positive predictive value, negative predictive value, cutoff values (CV) and precision using MRS coefficients in peritumoral edema for discrimination of high-grade gliomas and metastases using ROC curve analysis

Parameter	CV	Sensitivity %	Specificit y%	PPV%	NPV%	AUC	Precision
Cho/Cr	1.18	89.79%	93.33%	95.65%	84,.4%	92.2%	91.13%
Cho/NAA	1.115	93.87%	93.33%	95.83%	95.83%	95.8%	93.67%
NAA/Cr	1.155	67.34%	93.33%	54.09%	11.11%	16.3%	44.30%

4. DISCUSSION

Solitary metastatic lesions are virtually indistinguishable from high-grade gliomas on basic MRI sequences. Carrying out this differential diagnosis preoperatively is important both for surgical planning and for follow-up and complementary treatment modalities. Some patients with poor general condition or with many comorbidities could benefit from the absence of a biopsy, since the performance of this technique often involves the repetition of the procedure. MRS is a technique that provides semiquantitative in vivo information on the metabolic composition of different CNS lesions [6,7,1a]. The measure-ment of the different metabolites allows not only the differentiation of intracranial lesions, but also their tumor grading as well as assessing tumor viability and the state of the cell membrane and its turnover [8]. The spectroscopic pattern present in tumor lesions is characterized by a decrease in NAA with an increase in Cho levels, and lipids and lactates can be found in the tumor center due to necrosis or areas of ischemia produced by its rapid growth [6].

In our study, both the Cho/Cr and Cho/NAA coefficients obtained in peritumoral edema were statistically different between both pathologies [9,10]. These differences in the peritumoral area associated with each tumor type can be explained by its different pathophysiologies [11, 12, 1a]: abnormalities in the tumor endothelium, secretion of the tumor vascular growth factor, tumor angiogenesis, etc. In high-grade gliomas, the areas of peritumoral tumors show, in addition to morphologically altered capillaries and interstitial water, infiltrating tumor cells along preexisting dilated vascular channels and those formed de novo. In metastatic edema, the edema is purely vasogenic, consisting of fluid without tumor cells, a fact that does not occur in high-grade glioma edema [13, 14]. It should be noted that the different behavior between high-grade and metastases also conditions an increase in Cho at the peritumoral level at the tumor level (prima necro-sis). Cho is a faithful marker of the increase in membrane synthesis and therefore of cell multiplication [15,16]. The Cho/Cr index and the Cho/NAA are markers of degree of malignancy [17]. We have based our study on these differences in peritumoral behavior [1a].

Our findings are consistent with previous studies, observing a higher Cho/Cr ratio in peritumoral edema when comparing high-grade gliomas versus metastasis [18, 19]. Among the most important studies, the one carried out by Server et al. [20] in

2010 with a significant difference was obtained in Cho/NAA, Cho/Cr and NAA/ Cr coefficients in peritumoral edema between high-grade gliomas and metastases. In the same way, in the article by Weber et al. [21] an elevation in Cho/NAA ratios in the hyperintense perifocal T2 region was reported in high-grade gliomas, in contrast to the lack of elevation in metastases [1a].

Regarding the use of other indices, two renowned authors such as Chiang et al. and Law et al. [17, 18] did not find a statistical difference in the peritumoral analysis of the NAA/Cr coefficient between high-grade gliomas and metastases, a validated result, by our study [1.a]. This absence of difference between the two pathologies may be due to the absence of neuronal replacement or destruction in peritumoral areas. In high-grade gliomas, tumor cells infiltrate along the vascular channels but do not destroy the preexisting cytoarchitecture [22]. On the other hand, vasogenic edema associated with metastasis is a passive process that does not necessarily destroy the underlying structure or neural tissue [23]

We must not forget that N-acetyl aspartate or NAA is a neuronal marker and creatinine or Cr is a molecule that plays an important role in the maintenance of energy-dependent systems in brain cells. A low NAA/Cr coefficient is a reliable indicator of malignancy, and this decrease is also quantifiable with significant differences between gliomas of different grades [24].

Other authors have performed the spectroscopic analysis intratumorally rather than peritumorally. In our study, we have decided to do the analysis with the placement of the diagnostic voxel within the peritumoral area because it has been shown that it is less critical and that the degree of mixtures of the different types of tissue does not have significant implications for the calculated measurements, not being the case tumorally [1a]. Nelson et al. [25] in their study that it is difficult to obtain valid intratumoral spectra without partial tumor volume and necrosis due to the effects of tumor heterogeneity.

Regarding the presence of the lipid peak (tumor necro-sis reflex), there are several studies [26, 27] that reflect its existence both at the level of high-grade gliomas and metastasis due to the generation of tumor necrosis. This is in line with our results as we did not find significant differences. In another more recent study, they have found that the signal of a lipid macromolecule allows significant discrimination between both pathologies [28].

In our study, sensitivities of 93.87% and 89.79% were found for the Cho/NAA and Cho/Cr ratios together with area under the curve of 0.958 and 0.922, respectively. This allows us to deduce that given the sensitivity and the area under the curve, the Cho/NAA parameter is more discriminative, although both parameters have very good discrimination. In the literature, different sensitivity values are shown according to the cutoff number value [1a]. Against our cutoff point of> 1.114 for Cho/NAA (S: 93.87%), Al-Okaili et al. [29] in 2007 obtained a cutoff point of > 1 (S: 83.33%); Cai et al. in [30] obtained a cutoff point of> 1.085 (S: 93.75%) and finally in 2010 it was Server et al. [20] and Wong et al. [31] who obtained cutoff points of> 1.11 (S: 91.11%) and > 1 (S: 88.88%), respectively. Given these data, we can say that our analysis goes in the same direction as the literature [1a].

Table 6. Comparison of the different cutoff points in the literature providing means of sensitivity, specificity, positive predictive value, negative predictive value and precision

	Cho/NAA		Cho/Cr		NAA/Cr	
Study	**CV**	**Sensibility**	**CV**	**Sensibility**	**CV**	**Sensibility**
Our study	> 1.114	93.87%	> 1.17	89.79%	> 1.154	67.34%
Al-Okaili et al. [19]	> 1	83.33%	–	–	–	–
Cai et al. [20]	> 1.085	93.75%	> 1.032	81.21%	–	–
Server et al. [11]	> 1.11	91.11%	> 1.21	88.88%	> 1.17	68.88%
Wong et al. [21]	> 1	88.88%	–	–	–	–
Chawla. S et al. [22]	–	–	> 0.4	NA	–	–

Regarding the analysis of the Cho/Cr coefficient in the literature, compared to our > 1.17 with S: 89.79%, we found Cai et al. in 2009 [30] with a cutoff point of> 1.032 (S: 81.21%) and Server et al. and Chawla S. et al. [32] in 2010 with cutoff points of> 1.21 (S: 88.88%) and > 0.4 (S: not reported), respectively. We clearly realize that the sensitivity of this coefficient is less than that of Cho/ NAA, which in our opinion is the one of the choices in the first instance.

Finally, making a review of the NAA/Cr coefficient values in the literature, we find that compared to our 1,155 (S: 67.34%) Server et al. [20] provide a coefficient of 1.17 with a sensitivity of 68.88% [1a]. As we have previously explained, this coefficient is more effective in detecting metastasis vs gliomas than applying it the other way around. And this logical conclusion is also described in the literature. All this can be visualized in Table 6 [1a].

In addition to alterations at the level of metabolite coefficients, perfusion measured by rCBV at the peritumoral level also shows important differences between high-grade gliomas and metastases. In our study, we have carried out a qualitative study of perfusion; however, other authors such as Law et al. [19] have quantified the increase in perfusion up to 1.39 higher than normal white matter in contrast to perfusion in metastasis which was 0.39 (less than normal white matter).

At present, there are already published studies with diagnostic strategies based on advanced resonance techniques [33]. In this strategy, they combine spectroscopy, diffusion and enhancement of the lesion to make a first approximation. The final differentiation between high-grade gliomas and metastases is based on a Cho/ NAA coefficient greater or less than 1 [1a]. The global precision of this study estimates that with combined imaging techniques the diagnosis can be reached preoperatively in 85–90% of intraaxial lesions.

Our study has a limitation, which is found in all the studies with spectroscopic analysis, which is the effects of tumor heterogeneity whereby the metabolite signal can be influenced in some cases by the partial effect of volume.

5. CONCLUSION

In conclusion, our study shows that through advanced spectroscopy techniques the differentiation between high-grade gliomas (GBM and AA) and metastases, especially when measurements of the Cho/Cr and Cho/NAA ratios are included in peritumoral edema, is possible with high precision [1a]. In this case, a coefficient greater than 1.59 in the Cho/Cr ratio and 1.48 in Cho/NAA orients the diagnosis of high glioma vs metastasis with very high sensitivity and specificity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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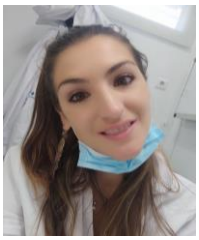
Biography of author(s)



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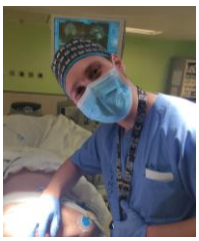
She is a specialist doctor who works in a prestigious hospital such as the San Carlos Clinical Hospital. Her areas of sub-specialization include tumor pathology, skull base surgery, and spine surgery. Her areas of research and publications have covered almost all fields of Neurosurgery from temporomandibular joint pain treated by peripheral stimulation to tumor spectroscopy in high-grade gliomas as a diagnostic and therapeutic method. She also has extensive experience in functional neurosurgery, particularly in Parkinson's disease. She carried out a study to highlight the network system involved in this pathology together with Dr. Torres. Treatment using deep brain stimulation stimulated this network system, causing changes at multiple brain levels. She has more than 100 courses in her specialty and over 70 communications at conferences in addition to numerous published articles. She is a researcher who represents a young promise given her research capacity and the facilities provided by the San Carlos Clinical Hospital.



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He is a specialist doctor from a prestigious hospital who performs his training in Neurosurgery in a formidable manner. Currently, his areas of interest include tumor pathology, skull base to-pain pathology, and spine surgery. In the same way, his research focuses on a totally innovative topic such as preoperative radiotherapy in high-grade tumors as a method of achieving a supramaximal resection and in this way getting closer to the end of curing the tumors. But this topic is not the only one to which he devotes attention. He has also published cases of novel surgeries in the field of pain and actively participates in communications at national and international conferences. His learning has also been based on multiple national and international courses. Given the high volume of patients treated at the hospital, he has managed to acquire an enviable surgical capacity and an excellent range of knowledge and indications in the different subfields of the specialty that together make him an exceptional doctor.

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He is an excellent neurosurgeon and researcher who focuses his work in the fields of pain, spine, and microsurgery. He is a doctor who practices his work at the highest surgical level. In relation to his research activity and publications, he is currently working on his doctoral thesis on the recovery of functions in patients with heart attacks through the transfer and local injection of stem cells. This field belongs to regenerative medicine, a booming field that is still unexplored, and together with Dr. Barcia, they are unraveling and obtaining excellent results. Another of his fields of research is spinal endoscopy, in which he is one of the best, given his innovative capabilities as well as the results obtained. He also participates in the publication of multiple case reports. Every year, he is invited to multiple conferences and teaches numerous courses. He carries out his work at the San Carlos Clinical Hospital, combining it with his work at the Ruber Clinic, a famous international entity.

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Chapter 3
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A Mini-Review of Last Guidelines about Management of Eosinophilic Oesophagitis

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ABSTRACT

Background: Eosinophilic oesophagitis is a chronic oesophageal disease that is driven by the immune system. It is defined histologically by mostly eosinophilic inflammation and clinically by symptoms resembling oesophageal dysfunction. Allergens have a significant part in causing this illness. Being an immunologically active organ, the oesophagus can draw in eosinophils in reaction to a variety of triggers.
Aim: The present study highlights about last guidelines about management of Eosinophilic Oesophagitis.
Methods: Numerous nations outside of Africa have recorded cases of eosinophilic oesophagitis, with men between the ages of. 20 and 30 and city dwellers having a higher frequency. There are several disorders linked to this problem, the most significant of which being gastroesophageal reflux disease (GERD).
Results: From a paraclinical point of view, patients have a peripheral eosinophilia, and diagnostic certainty is realized by performing upper endoscopy with biopsy. The recommended treatment has 3 stages, namely diet, drug therapy (such as fluticasone propionate, budesonide and proton-pump inhibitors) and investigations such as upper endoscopy.
Conclusion: The article aims to highlight recent recommendations in international guidelines for the management of eosinophilic oesophagitis, as well as to review its clinical manifestations, genetics, immunopathogenesis diagnosis and treatment. A deeper understanding is needed to inform clinical decisions regarding optimal disease follow-up and the use of long-term maintenance therapy.

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Keywords: Eosinophilic oesophagitis; gastroesophageal reflux disease (GERD); peripheral eosinophilia; upper endoscopy with biopsy; international guidelines.

1. INTRODUCTION

Eosinophilic oesophagitis is a chronic inflammatory disease characterized by eosinophilic infiltration of the oesophageal mucosa. Food and aero-allergens are involved in its pathogenesis. Dysphagia and food impaction are the dominant symptoms in adult with eosinophilic oesophagitis. However, a wide range of symptoms has been noticed such as chest pain or gastro-oesophageal reflux disease-like symptoms [1]. When gastrointestinal eosinophilia is limited to the oesophagus, and accompanied by characteristic symptoms, other causes of eosinophilia are excluded. In that case, the term used is eosinophilic oesophagitis [2]. Seasonal exacerbations of the described charges have suggested a possible role of aeroallergens such as pollen seasons, which contribute to eosinophilic oesophagitis through a regional or systemic response [3]. The mechanism of food allergy (wheat, cow's milk,egg, shellfish, nuts, soy) can vary from cell-mediated hypersensitivity to chronic autoimmune reactions [4].

Epidemiologically, eosinophilic esophagitis has been reported in the USA, Europe, Asia and Australia. This disease is not present in Africa [5]. Increased incidence has been observed in urban areas [6], with the majority of affected adults being men aged between 20 and 30 [7,6a]. The first cases of eosinophilic oesophagitis were reported between 1960 and 1970 [8].

Recent studies have shown that smoking, long-term administration of NSAIDs [9] and the presence of Helicobacter Pylori [10] do not present statistically significant risk factors, but antibiotic therapy and antacid medication have a high potential risk to lead to the development of this condition [11,12, 6a].

The pathogenic mechanism of eosinophilic oesophagitis has not been studied, but the implications of genetic, environmental and immune factors are recognised. The oesophagus of patients with this condition have an imbalance between epithelial cells and the 'barrier' function, thus leading to the appearance of oesophageal epithelial dysfunction [6a]. The mechanism of the immune system also shows an imbalance due to the mismatch between Ig E mediated response and the delayed response of helper T cells type2 (Th2), which produces cytokines, being an activation factor that primes eosinophils and prolong their cellular survival [13]. The pathogenesis of eosinophilic oesophagitis involves a number of allergens, cytokines, microRNAs [14] and chemokines (IL4, IL-5, IL-13, eotaxin -3). At the same time, several genetic defects have been identified that predispose patients to the onset of eosinophilic oesophagitis, namely the deletion of chromosome 2p23 and calpain-14 (calcium-dependent cystein protease) [6]. The most strongly specific gene associated with eosinophilic oesophagitis is eotaxin -3 which recruits eosinophils from the peripheral blood [15]. The genes for Thymic stromal lymphopoietin (TSLP) promots Th2 responses and has elevated levels in the patients with eosinophilic

oesophagitis [16]. Other genetic variants which are associated with eosinophilic oesophagitis are desmosome and filaggrin genes, the latter one has a role of alterations in barrier function, being related to inherited connective tissue disorders [17].

2. DISCUSSION

The clinical manifestations of eosinophilic oesophagitis vary with age [18], as follows: adolescents and adults frequently have dysphagia and food impactation, while children more often report symptoms of gastroesophageal reflux disease and abdominal pain [6a].

The most common clinical manifestations in adults are:

- Dysphagia - the most common symptom [19,20]
- food impaction, present in approximately 50% of patients [21,22]
- Chest and retrosternal pain that does not respond to antacid therapy
- Treatment-related heartburn and other symptoms associated with GERD [23,6a]
- Disorders of oesophageal motility suggesting involvement of oesophageal muscle layers [24]

Eosinophilic oesophagitis is associated with other allergic conditions, such as:

- Asthma [25]
- Chronic rhinosinusitis [26]

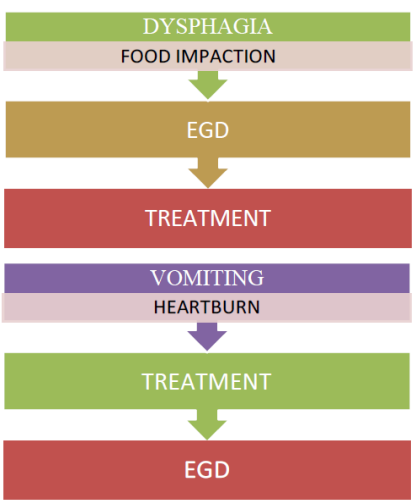

Fig. 1. Confirmed oesophageal eosinophilia

- Atopic dermatitis [27]
- Celiac disease [28,29]
- Inflammatory bowel disease [30]
- Exposure to caustic agents [31]
- The Schatzki ring has been described, but the association has not been sufficiently investigated [32]
- Allergic vasculitis
- Parasitic or fungal infections
- Neoplasms

The definite diagnosis of eosinophilic oesophagitis is based on the association of chronic symptoms of oesophageal dysfunction, personal history of allergic diseases, personal history of pathology (parents who have been suspected of eosinophilic oesophagitis), history of postoperative oesophageal perforation, confirmed by upper endoscopy with biopsies and laboratory tests [6a].

The diagnostic criteria for eosinophilic esophagitis are as follows [33]:

- Symptoms associated with oesophageal dysfunction.
- Predominantly eosinophilic inflammation highlighted on biopsy (≥ 15 eosinophils per high power field (HPF) or 60 eosinophils per mm2).
- Exclusion of other causes that may contribute to the onset of eosinophilic oesophagitis [6a].

The characteristics of upper endoscopy highlight the following [34]:

- Fixed (corrugated or trachealised) and transient oesophageal rings (feline folds or felination = stacked circular rings), strictly common in the proximal region
- Attenuation of the subepithelial vascular pattern
- Linear furrows
- Whitish papules (eosinophilic microabcesses)
- small-calibre oesophagus

Biopsies should be taken from both the proximal or middle level (2-4 biopsies) and the distal level (2-4 more biopsies) [6a,35].

The histological aspects of eosinophilic oesophagitis include the following:

- Sheets of eosinophils
- Eosinophilic microabscesses
- Superficial layering of eosinophils
- Extracellular eosinophil granules [36]
- Basal cell hyperplasia
- Enlarged intercellular spaces
- Epithelial exfoliation
- Subepithelial and lamina propria fibrosis [37]
- Large number of mast cells and B cells [38,39]
- Papillary lengthening

Table 1. Treatment in eosinophilic oesophagitis

Proton pump inhibitors (PPIs)	**Topical glucocorticoids**			
• First-line treatment options • once daily/twice daily for eight weeks [45] • control the symptoms • reduce acid production in patients with coexistent GERD	**Fluticasone propionate** • dose inhaler without a spacer – 220 mcg/spray , 4 sprays daily in divided doses for 4-8 weeks [46] • symptoms improvement • oesophageal eosinophilia is not improve • decrease eosinophilic counts • side effects: oesophageal candidiasis, herpes esophagitis, cataracts (at higher doses) [47], adrenal suppression • higher rates in sustained remission [49]	**Budesonide** • orodispersible tablet for adults or oral viscous slurry – 2mg daily for adults [48] for 12 weeks [45] • symptomatic improvement • it is effective for treating eosinophilic esophagitis • decrease eosinophilic counts • approved by European Medicine Agency and Health Canada • higher rates in sustained remission [49]	**Ciclesonide** • 80 or 160 mcg, 2 sprays twice daily for 2 months [50] • symptomatic improvement • eosinophilic esophagitis improvement • further studies are needed	**Mometasone furoate** • treating children and adolescents with eosinophilic esophagitis[51] • further studies are needed

Oesophageal dilatation is effective in relieving symptoms like dysphagia patients with high-grade strictures or rings who have not responded to pharmacological therapy [52]

Some studies highlight that endoscopic and histologic findings are modestly predictived of symptoms severity, but there are incompletely studied [40]. A scoring system is developed for establishing diagnosis of eosinophilic oesophagitis, named Index of Severity for Eosinophilic Esophagitis (I-SEE) which assessed symptoms and complications, endoscopic and histoloic features, with a point value ranging from 1 to 15 [41].

Barium transit is not a gold standard for eosinophilic oesophagitis, but it can provide information on strictures [42]. Endoscopic ultrasound, oesophageal manometry and impedance planimetry to measure oesophageal pressure and distensibility are not routinely performed in cases of eosinophilic oesophagitis [6a,43].

Paraclinically, patients have eosinophilia in the peripheral blood> 300-350/mmc associated with elevated Ig E levels (> 114000U/L). Allergic tests are used to identify comorbidities such as asthma, allergic rhinitis, etc., skin prick testing (SPT) and atopy patch testing (APT) are methods of testing food allergies [44].

Table 2. Experimental versus ineffective treatments in eosinophilic oesophagitis

Experimental	Ineffective
Monoclonal antibody against IL-13	
● Significant reduction in oesophageal eosinophil count, endoscopic severity and histological grade ● No significant improvement in dysphagia symptoms [53] ● **Has been implicated as an important cytokine in the pathogenesis of eosinophilic oesophagitis [53]**	
Dupilumab ● A monoclonal antibody to the α subunit of the IL-4 ● Improvement in dysphagia, histologic and endoscopic findings [54] ● **Inhibits signaling of IL-4 and IL-13 cytokines, which is important in the generation of inflammation mediated by T helper type 2 (Th2) cells** ● **May be best reserved for patients who are refractory to or decline other options [55]**	
● **Mepolizumab** A humanised monoclonal antibody against IL-5	● Anti-TNF = Infliximab

Experimental	**Ineffective**
● Significant role in eosinophil recruitment [56]	● Antihistamines and cromolyn
Reslizumab	
● IL-5 neutralising antibody ● Significant reduction in eosinophil counts [57]	● Anti –Ig E monoclonal antibody
Prostaglandin D2 receptor antagonist ● Significant reduction in eosinophil counts ● Symptom improvement [58] ● **Is a receptor expressed by Th2 cells, eosinophils, and other inflammatory cells that mediates chemotaxis of these cells in response to prostaglandin D2 [58]**	
Montelukast ● Maintenance of remission [59] ● **A leukotriene inhibitor** ● **Symptoms reduction [59]**	
Purine analogues ● Clinical and histological improvement [60]	

From dietary point of view, the four-food (wheat, cow's milk, egg, shellfish, nuts and soy) empiric elimination diet followed for 8 weeks represents the best approach for most patients. After the avoidance period, an upper endoscopy with oesophageal biopsies is performed, and foods are reintroduced one at a time [6a,61].

The long-term prognosis of eosinophilic oesophagitis is unclear, but some studies revealed that the disease may progress to a fibrostenotic form (no cases of malignancy were observed) in which intermittent dysphagia represents the most important symptom [62]. Patients underwent a follow-up examination consisting of laboratory testing and an upper endoscopy with biopsies.

American Society for Gastrointestinal Endoscopy Consensus Conference highlight an Endoscopic approach to eosinophilic esophagitis statements: 1." In patients with fibrostenosing EoE, dilation therapy should occur in conjunction with effective medical or diet elimination therapy for management of dysphagia". Several lines of evidence suggest that the need for repeat dilation in EoE patients after an initial dilation was lower in patients on maintenance therapy. Cessation of medical therapy after achievement of histologic remission leads to esophageal caliber decreased and symptom recurrence.

2." In patients with fibrostenosing EoE with inflammatory activity, dilation can be done safely." Several systematic reviews all have demonstrated that the rate of serious adverse events is very low [63].

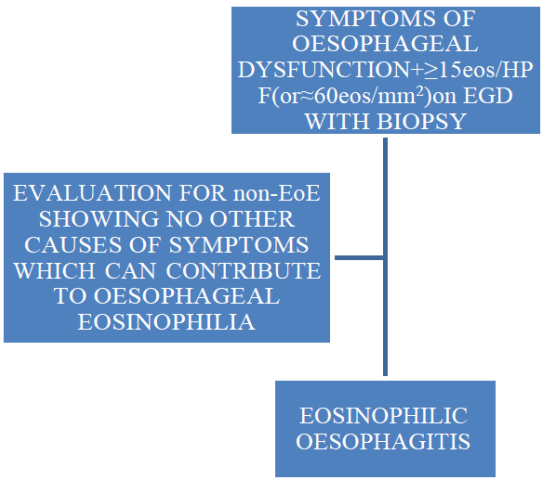

Fig. 2. Diagnostic algorithm for patients with eosinophilic oesophagitis [64]

The first diagnostic guidelines on eosinophilic oesophagitis were published in. 2007 and updated in. 2011 [64]. This condition was characterised as a distinct clinical entity by Atwood and Straumann in the early 1990s [6a,65].

Many patients with clinical symptoms and oesophageal eosinophilia ≥15eos/HPF who responded to treatment with high-dose PPIs, but did not have manifestations of GERD, were defined as having PPI-responsive oesophageal eosinophilia (PPI-REE) according to guidelines published in 2011,. 2013 and. 2014 (64). Oesophageal eosinophilia and GERD are distinct conditions, but they can coexist: the first one can lead to reflux due to oesophageal dysmotility and the second can decrease epithelial barrier integrity, leading to eosinophilia [66,67].

Patients with symptoms of oesophageal dysfunction and ≥15 eos/ HPF (or ≈60 eos/mm2) on biopsy are defined as having suspected oesophageal eosinophilia (PPIs can be used when there is a histologic improvement), while those with symptoms of oesophageal dysfunction and ≥15 eos/HPF (or ≈60 eos/mm2) on biopsy after evaluation for other causes of the condition are characterised as having confirmed oesophageal eosinophilia [6a].

AGA Institute and the Joint Task Force process for developing clinical practice guidelines recommend the following for patients with eosinophilic oesophagitis:

1. Topical glucocorticosteroids over no treatment.
2. After short-term use of topical glucocorticosteroids, which leads to remission, the continuation of this treatment over discontinuation of therapeutic scheme
3. IPPs for symptomatic conditions over no treatment
4. Elemental diet using an empiric six-food elimination diet

5. Allergy testing-based elimination diet [6a].
6. Endoscopic dilatation for all cases of dysphagia from a stricture
7. Using anti IL-5, anti-IL-13, anti – IL-4 receptor-α, anti –Ig E therapy, montelukast, cromolyn sodium, immunomodulators, and anti-TNF only in the context of clinical trials.

The recently published European and International consensus statements have removed the PPI trial from the diagnostic criteria for eosinophilic oesophagitis. The exclusion of a PPI trial is that patiens with eosinophilic oesophagitis and initial response to PPIs could develope recurrent symptoms and those who respond histologically to a PPI are considered to be included in a subset of this condition rather than a distinct disease [33,68].

3. CONCLUSIONS

In the last. 20 years, studies and clinical trials have provided an evidence base that highlights the progress which has been made in the understanding of eosinophilic oesophagitis from clinical, treatment and management strategy points of view [6a]. On the other hand, there are many unknowns and controversies regarding this condition. A deeper understanding is needed to inform clinical decisions regarding optimal disease follow-up and the use of long-term maintenance therapy.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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The Effect of Environmental Factors on the DNA Methylation Pattern and Its Impact on Addiction

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ABSTRACT

Substance addiction remains a significant social and medical issue, influenced by both environmental and genetic variables. The interplay between genetic and ecological factors demonstrates the importance of the epigenetic mechanism. Given its ability to control and influence enduring alterations in gene expression, epigenetic regulation provides a compelling hypothesis for the persistent behavioral abnormalities associated with drug addiction. Epigenetic mechanisms, including DNA methylation, significantly influence drug addiction and substance abuse. Environmental factors such as parental influence, cultural norms, media representation, and learned physical associations also contribute to addiction. Nutritional, pharmacological, physical, and psychosocial factors (such as stress exposure) also affect the DNA methylation profile, leading to changes in gene expression and vulnerability to drug addiction. Studies show that genetic predispositions to psychiatric illnesses like addiction vulnerability can be attenuated or regulated by environmental factors. This chapter will focus on the effect of DNA methylation and environmental factors on drug addiction. Understanding these variables could potentially provide interpretive tools for the future diagnosis and management of addiction.

Keywords: Epigenetics; drug addiction; DNA methylation; environmental factor.

1. INTRODUCTION

Substance addiction, which includes alcoholism and illegal drug use, is a severe issue marked by obsessive drug seeking and persistent hazardous use despite detrimental effects [1]. Addiction rises from a confluence of hereditary and

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environmental elements that interact to create a cumulative, irreversible, and lifelong effect, much like other complicated illnesses [2]. Addictive drugs stimulate the brain's natural reward system through the release or synaptic accumulation of the neurotransmitter dopamine [3,4]. More importantly, the genesis of drug addiction is the result of several environmental variables coming together (like real exposure to illicit drugs) and inherited predisposition (such as genetic polymorphisms influencing the characters related to drug-seeking behavior and dependency). Research on families, twins, and adoptions suggested that the heritability of drug addiction is contributed from moderate to high (ranging from 30 to 70 percent) by shared and non-shared surrounding factors [5]. Numerous evidence suggest that gene expression occurs in abnormal patterns in different regions of the brain related to drug addiction, such as the prefrontal cortex, the nucleus accumbens, the amygdala, and the ventral tegmental area [6,7,8]. The interaction of genetic and environmental factors signifies the role of epigenetic mechanisms.

Epigenetics encompasses the biochemical modifications responsible for changes in gene expression throughout an organism's lifecycle, independent of alterations in the DNA sequence. This includes reversible modulation of gene expression primarily driven by DNA methylation and histone modification [9]. Various epigenetic mechanisms, including DNA methylation, histone modifications, and chromatin rearrangement, play a crucial role in the long-term regulation of gene expression, serving as a molecular memory for genes and their interactions with the environment [10]. Researchers have demonstrated that epigenetic variables have a significant role in the vulnerability to specific common neuropsychiatric phenotypes associated with addiction, such as schizophrenia [11] and depression disorders [12]. Other evidence also suggested that long-lasting neuroplasticity changes observed in drug addiction may be partially regulated by epigenetic alterations [13]. Numerous findings indicate that exposure to external environmental factors has the potential to impact epigenetic modifications globally or at specific genomic loci [14]. Given that epigenetic modifications are transmitted mitotically in somatic cells, they present a potential mechanism through which the influence of external environmental stimuli at specific developmental stages can lead to enduring behavioral changes. Of particular interest in the context of addictive behavior, there is a wealth of evidence suggesting that drugs of abuse directly influence epigenetic processes, and these alterations may contribute to the molecular underpinnings of addiction. DNA methylation is viewed as a more enduring epigenetic modification compared to histone tail modifications, which are considered more readily reversible.

Gene expression can alter over time due to the mitotic stability of DNA methylation patterns. However, these patterns also exhibit a discernible degree of adaptability over time, allowing cells to adapt to shifting internal and external influences [15]. Research has shown that DNA methylation status also regulates the neuroplasticity brought on by abusing drugs, and it may have a significant impact on the onset and symptoms of drug addiction [16,17]. A consistent adaptation, in line with the persistent and experience-driven nature of addiction, involves the lasting epigenetic changes to the DNA of affected neurons [7].

Through conformational alteration to chromatin shape and accessibility, these alterations affect transcription without directly changing the nucleotide sequence. These changes can affect underlying transcription levels of genes differently, priming/desensitizing particular genes for stimulation or suppression in response to drugs, or triggers or regulating splice variant expression [8,9]. Since addiction does not develop until after repeated exposure to an addictive substance, the causes of addiction can only be fully understood by identifying the inherited and environmental predisposing variables along with the dynamic neurobiological alterations brought on by long-term drug exposure.

2. DNA METHYLATION AND ADDICTION

DNA methylation is the process of introducing a methyl group to the C5 position of cytosine (5-mC) [18]. DNA can be modified by an enzyme called DNA methyltransferases (DNMTs), which facilitate the addition of a methyl group to the cytosine and is traditionally observed at cytosine-guanine dinucleotide (CpG) residues [9]. DNA methyltransferase 1 (DNMT1) maintains DNA methylation through the replicative process, which copies an existing 5mC onto the complementary DNA strand after cell division [19]. DMNT3a and DMNT3b are considered de novo methyltransferases, responsible for methylating previously unmethylated cytosines to establish a pattern of DNA methylation [20,21]. The identified forms of cytosine modification can return to an unmethylated state through base excision repair, glycosylation, or deamination. In addition to other functions, DNA methylation is necessary for healthy organismal development, genetic imprinting, X chromosome inactivation, and tissue-specific gene expression [22]. DNA methylation is believed to be a gene expression repressive modification. A group of proteins translates DNA methylation status to gene regulation by attracting regulatory complexes to promoters and enhancers. Methylation at particular cytosine-guanine dinucleotides (CpG islands) impedes transcription factors binding to target regions by assembling multiple corepressor complexes. MeCP2 and MBD1 are methyl-binding domain-containing proteins that attract repressive complexes to methylated DNA. These proteins also stabilize other corepressors, such as HDACs, near gene promoters. DNA methylation at gene promoters can impact the transcription factor CREB's binding because the consensus cAMP response element (CRE) sequence has a CpG island that, when methylated, inhibits CREB from binding to its target sequences [23]. The 5-hydroxymethylcytosine moiety might be an epigenetic mark different from 5-methylcytosine [24,25].

Increasing research has started to elucidate the role of DNA methylation in drug addiction. Furthermore, studies have shown that the enzyme DNA methyltransferase 3a (DNMT3a), plays a direct function. A decrease in DNMT3a activity in nucleus accumbens causes increased behavioral reactions to cocaine, which can be achieved through either pharmacological suppression or viral-mediated enzyme knockdown [26]. Researchers discovered aberrant DNA methylation patterns in the promoter regions of several genes in both drug and alcohol addicts as well as animal models. Furthermore, they demonstrated a significant correlation between DNA methylation and alcohol and nicotine

dependency in women, particularly at the monoamine oxidase-A (MAOA) gene [27]. Another discovery revealed that heroin users' blood and brain tissue had increased levels of methylation at CpG-rich islands within the opioid receptor gene (*OPRM1*) [28,29,30]. Compared to individuals who are not taking opioid painkillers, researchers have shown increased methylation levels in patients undergoing long-term treatment, suggesting that prolonged opioid exposure has pharmacological effects [28]. A study also indicated increased DNA methylation at the *OPRM1* (opioid receptor mu 1) gene promoter of methadone-maintained former heroin addicts, leading to a decrease in *OPRM1* gene expression in lymphocytes [29]. It is possible that hypermethylation of CpG sites in the *OPRM1* promoter may block the binding of Sp1, as well as other transcription factors, leading to *OPRM1* silencing. Blood samples from alcohol-dependent people also showed higher levels of DNA methylation in the HTR3A serotonin receptor gene [31]. A finding concluded that the severity of alcoholics' drinking patterns correlated negatively to DNA methylation of a cluster of CpGs associated with the *NR2B* (NMDA receptor 2B) gene promoter region [16]. In a separate study, researchers identified hypermethylation of the Arginine Vasopressin (*AVP*) gene associated with alcoholism. Conversely, DNA hypomethylation was observed in the promoter region of the atrial natriuretic peptide (*ANP*) gene [32]. Additionally, researchers demonstrated that inhibiting DNMT activity led to alterations in the methylation profiles of the *reelin* and *BDNF* promoters—two genes linked to the modulation of synaptic plasticity in the adult hippocampus [33]. Thus, drugs and alcohol exposure induce hypermethylation or hypomethylation in gene promoter regions, that affect the gene expression. The general consensus is that DNA methylation represses gene transcription by attracting co-repressor complexes (such as HDACs and HMTs), which can alter the structure of nucleosomes or sterically impede the transcriptional machinery. This leads to the individual being unable to attain sufficient feelings of reward in the absence of more drugs. Such complexes involve several DNA methyl-binding domain proteins (MBDs) [34]. When methylated CpG dinucleotide binds to methyl-binding domain (MBD) proteins, it promotes histones to become deacetylated, resulting in chromatin to condense and genes to be repressed [35]. The activation of the brain's reward circuitry is believed to be a common mechanism across different forms of addiction. This circuitry is centered on dopaminergic neurons in the midbrain's ventral tegmental area (VTA) and their projections to the limbic system, specifically the nucleus accumbens, also known as the ventral striatum, dorsal striatum, amygdala, hippocampus, and areas of the prefrontal cortex. [36,37,38]. Collectively, these findings underscore the effects of drug usage on gene expression through DNA methylation.

3. ENVIRONMENTAL FACTOR AND ADDICTION

Environmental factors such as peer pressure, health problems, family conditioning, and physical, emotional, and sexual abuse can additionally have an impact on addiction, despite the fact that multiple genes have been associated with the phenotypes of addiction [39]. For example, urban locations, which have a higher proportion of juveniles and illicit drug sales than rural settings, exhibit an approximately five-fold increase in the genetic impact on alcohol intake [40].

Results imply that exposure to external environmental influence could affect epigenetic processes globally or at particular loci [41]. Primary research and experiments on DNA methylation changes that occur postnatally could give some ideas on the fundamental principles that control the functions of epigenetics as an environmental mediator. DNA methylation profile varies due to nutritional, chemical, physical, and psychosocial factors (e.g., stress exposure). The role of epigenetic processes in modulating the phenotypic effects of environmental stimuli is supported by evidence that genetically similar animals and identical twins can experience epigenetic variation due to environmental factors [42,43]. According to a study, adolescents with lower socioeconomic status have higher levels of DNA methylation at the serotonin transporter gene's promoter region in blood cells, which predicts alterations in risk-related brain functions and makes these people more vulnerable to addiction [44]. Slight modifications in methylation have been observed in blood samples from both human and mouse offspring born to mothers exposed to alcohol consumption during pregnancy [45]. In another study, human abuse throughout childhood results in a reduction in gene expression and more methylation at the promoter region of the glucocorticoid receptor [46]. These alterations appear to be associated with relevant metabolic genes. Gene expression is downregulated as a result of methylation predisposition in genes associated with addiction. Once exposed to addictive drugs, this alteration enhances a person's susceptibility to addiction. By releasing dopamine or causing synaptic buildup of the neurotransmitter, addictive drugs activate the brain's natural reward system. As drug usage increases over time, dopamine-producing cells become more sensitive to impulses from surroundings linked to drug use, such as persons, places, odors, and images. Eventually, the signals trigger a dopamine response, stimulating the desire for the drug [47]. The excessively rewarding effects of drugs often override the more balanced dopamine released by natural rewards. Gradually, natural rewards decrease and the focus switches to achieving the elevated dopamine release generated by the drugs. As the brain adapts to elevated dopamine levels, tolerance to the drug starts to form, requiring higher dosages to produce the same level of euphoria which leads to drug addiction. In a significant study, specific genomic sites exhibited methylation changes in adipose tissue when mice were exposed to a high-fat diet, resulting in the development of obesity and other diabetes-like signs [48]. In addition to exhibiting particular DNA methylation patterns and retaining the germline molecular memory, imprinted genes also depend on epigenetic reprogramming following environmental exposures [49]. Gene-environment interactions, which happen when genetic variables interact with the environment to determine behavioral phenotypes, have been highlighted in recent studies. For instance, the 5-HTTLPR mutation in the promoter of the *SLC6A4* gene, which codes for the serotonin transporter and is a target of cocaine and 3,4-methylenedioxy-N-methylamphetamine (ecstasy), has been the subject of numerous investigations [50]. Serotonin transporter expression and serotonin uptake are both reduced by the short 5-HTTLPR allele [51]. For instance, participation in community-building activities or exposure to supportive parenting was found to mitigate the prevalence of substance addiction initiation in subjects with one or two copies of the short allele in an adolescent study [52]. Research like this shows that environmental factors can control or completely

minimize genetic predispositions to mental disorders such as addiction. The epigenetic regulator methyl CpG binding protein 2 (MeCP2) in the nucleus accumbens has been shown to be altered by both early life stress and methamphetamine, which influences the methamphetamine's motivating effects and natural reinforcement [53]. These fundamental studies provide important new information about how adverse environmental factors influence addictive behavior. Global DNA hypomethylation was also seen in blood samples from individuals with low socioeconomic status [54]. Thus, environmental interventions on the epigenome provide a mechanism for understanding gene-environment interactions implicated in neurological illnesses, including those underlying susceptibility to addiction [55].

4. CONCLUSION

DNA methylation and its surrounding influences play a pivotal role in drug abuse. However, a more efficient and reliable preclinical research approach, along with studies focusing on locus-specific alterations in DNA methylation, is needed to fully comprehend the intricacies of DNA methylation in drug addiction. Once identified, the genes impacted by DNA methylation can be cross-referenced with locations affected by histone modifications to gain a comprehensive understanding of the interactions between these drug-induced epigenetic alterations and the control of gene expression.

Chemical agents that modify DNA methylation may serve as effective candidates for medical treatment, given the dynamic and reversible nature of epigenetic systems. With a deeper understanding of the mechanisms behind the addiction process, researchers can develop potent pharmaceutical medications and diagnostic biomarkers for the treatment and prevention of addiction, relapse, and other neurological disorders.

DNA methylation profiling offers insights into an individual's environmental exposure history. The field of drug addiction and overuse epigenetics provides a molecular understanding of the issue. To achieve this goal, studies utilizing genomic technologies, such as whole-genome and gene-specific bisulfite sequencing, must ascertain how drug abuse affects DNA methylation modifications and gene expression.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Investigating the Dental Health and Oral Hygiene Status of Pregnant Women in Rural India

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ABSTRACT

The dental health is affected by increase in levels of oestrogen and progesterone hormone in the second month of pregnancy and continues to affect till term and subsides after puerperal period. There is increase in periodontitis, gingivitis and dental caries in women during pregnancy. The outcome of pregnancy is affected by increase in preterm delivery, low birth weight and preeclampsia in pregnancy. The outcome is influenced by life style, dental hygiene and socioeconomic status.

The cohort prospective study was carried out in the department of Obstetrics and Gynecology at Integra Institute of Medical Sciences and Research, Lucknow from March 2012 to April 2013, for the period of 1year. The dental health statuses of 600 antenatal cases were studied. The prevalence of gingivitis, dental caries and periodontal disease were studied. The association between poor oral hygiene, gingivitis and dental caries were studied in the pregnant women. In the study the prevalence of dental caries, gingivitis and periodontal disease were 90%, 98% and 90.33% respectively. The antenatal cases with poor oral hygiene were 2.5times more likely to have dental caries.

Keywords: Pregnancy; gingivitis; dental caries; periodontal disease.

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1. INTRODUCTION

The dental health is affected during pregnancy, breastfeeding and menopausal period. Pregnancy is associated with physiological changes which are demanding to women physically and emotionally and needs considerable support from family and health care system. Due to increase susceptibility and poor dental hygiene during pregnancy, adverse outcome like premature delivery, low birth weight baby, preeclampsia, gingival tissue ulcerations, pregnancy granuloma, gingivitis, pregnancy tumors, loose teeth, mouth dryness and dental erosions can occur. An old quote, “with each child, a tooth” holds place in dental and medical literature. The increase in circulating levels of estrogen and progesterone in second month of pregnancy and continuous to rise till eight month is reflected by increase in gingival inflammation during pregnancy. Increased level of progesterone cause increase capillary permeability and gingival exudates. Estrogen causes change in keratinisation of gingival epithelium. The anaerobic flora prevotella intermedia, bacteroides increase during 13th week of gestation and remain high in third trimester. Current evidence suggest an increase association between periodontal disease and increase risk of systemic disease atherosclerosis, myocardial infraction, stroke, diabetes mellitus and adverse pregnancy outcome including preterm birth, low birth weight, abortion and preeclampsia. The dental procedures in symptomatic patients may be deferred to second trimester or post delivery so that the organogenesis period and foetus during pregnancy is not exposed to anaesthetic and analgesic drugs. The Laser treatment may give good results as the Pyogenic granuloma has tendency to bleed. Some data have shown scaling root planning to pregnant women with periodontitis may reduce the risk of preterm birth [1,2].

1.1 Physiological Changes in Pregnancy

The oestrogen increases 10 fold and progesterone increases 30fold and systemic changes take place in cardiovascular, haematologic, respiratory, renal, gastrointestinal, endocrine, and genitourinary systems. The gastrointestinal changes involve increase intragastric pressure, reduction in the lower oesophageal sphincter tone due to increase in progesterone which causes decrease in production of motilin peptide hormone. The pregnant women experience heart burns, prolong gastric emptying, nausea and vomiting. Due to vena cava syndrome the pregnant women experience dizziness and fainting episodes. Hence morning dental appointments may be avoided during pregnancy and prolong sitting in dental chairs may be avoided. They may be made to lie down inclined to left or with hips slightly raised 10-12 cm in dental chairs [3,4].

The oral changes seen in pregnancy are dental caries (Fig. 1), gingival hyperplasia, pyogenic granuloma, salivary changes, worsening of periodontal disease, pyogenic granulomas (Fig. 2), plaque formation and gingivitis (Fig. 3). There is loose tooth or increase in mobility of tooth in late pregnancy which resolves in postpartum period. The gastric acids due to vomiting causes erosion of enamel. The remedial measures like frequent rinsing and sodium bicarbonate neutralise the acids. The routine brushing may be painful and there may be

bleeding from gums due to gingivitis. There is decrease in sodium, pH, increase in potassium, protein and oestrogen levels. Some studies show high oestrogen level in saliva is a indicator of preterm labour. Some studies show positive correlation between periodontal disease and preterm labour and low birth weight [5,6].

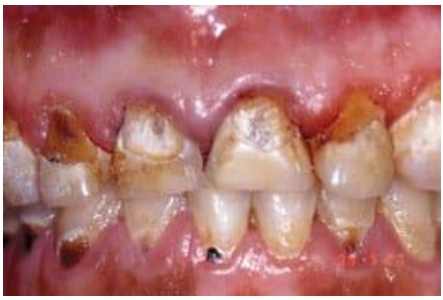

Fig. 1. Dental caries

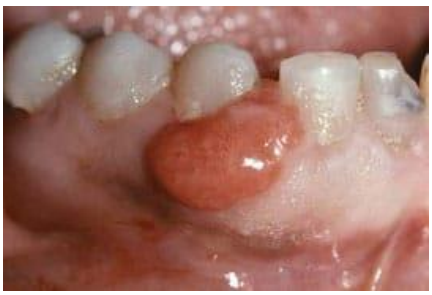

Fig. 2. Oral pregnancy tumor

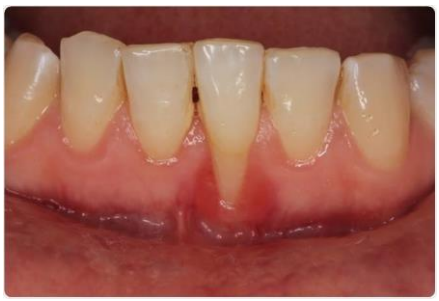

Fig. 3. Gingivitis

The radiation exposure can cause damage to foetal cells and cause miscarriage, birth defects. The teratogenicity depends on foetal age and dose of radiation. The critical period is 4-18 weeks of conception when the organogenesis may be

affected and more than 0.20 Gy can cause microcephaly and mental retardation. CT is useful in deep pharyngeal infections but it is to be replaced by MRI as an alternative in pregnancy due to high radiation exposure.

The drugs prescribed during pregnancy have to be according to FDA guidelines. Certain drugs can cause miscarriage, teratogenicity, low birth weight and should be used with caution during pregnancy and lactation [7-9].

2. METHODS

The Cohort Prospective study was carried out in the department of obstetrics and gynecology at Integral Institute of Medical Sciences and Research, Lucknow from March 2012 to April 2013 for a period of 1 year [9a]. The Institution caters to the medical and dental needs of rural population of central Uttar Pradesh. The antenatal cases in the first trimester attending the outpatient were counseled and after the consent were registered in the study. The detailed sociodemographic history of cases, age, parity, socioeconomic status, education, religion, obstetric history were taken. A detailed history of addiction to tobacco/pan was taken. The hygiene of the cases were assessed by the brushing habit (with brush/fingers). Use of tooth paste/gul(local ayurvedic preparation) as practiced in the community in the rural setup, was elicited. The sample was grouped into symptomatic and asymptomatic groups. All the cases were subject to routine dental examination on outpatient basis. The World Health Organisation criteria, 'newly developed cavity (dental caries) and gingival bleeding on probing (gingivitis)', was taken into consideration. The oral hygiene was evaluated by examination and questionnaire [9a].

The two components the debris/plaque (DI) and the calculus (CI) were used to calculate the oral hygiene index (OHI-S index=DI+CI). The OHI-S index <1 was considered good and OHI-S index ≥1 was considered poor. The questionnaire pertaining to dental health care included the knowledge, attitude and behavioral habits [9a]. The cases were further categorized depending on the oral disease into cases treated conservatively (medicine and oral hygiene) and cases treated by procedure (scaling) in second trimester and the untreated group. The prevalence of gingivitis, dental caries and periodontitis in pregnant women were studied along with the associated factors. The cases were subsequently followed up in each trimester, and the prognosis and response to dental care education, hygiene and treatment was evaluated [9a]. A logistical model was applied to calculate the odds ratio (OR) and 95% confidence interval (CI) of risk factors for dental caries and gingivitis. A p-value of <0.05 was considered statistically significant.

3. RESULTS

In the study, a total of 600 antenatal cases were studied for a period of 1 year. The subjects were in the first trimester [9a]. The history and the dental health status of cases were elicited by using a proforma. The cases were classified into symptomatic and asymptomatic cases. 86.7% of cases were symptomatic and

13.3% of cases were asymptomatic. The commonest symptom was bleeding from gums on brushing (84% of cases). Other symptoms were pain, loose tooth and sensitivity. The above symptoms were conspicuously noticed in the beginning of second trimester in 86% of cases and in 14% of cases symptoms were preexisting before pregnancy and got aggravated in the second trimester. 588 cases had gingivitis, 540 had dental caries and 542 cases had periodontal disease. The prevalence of dental caries, gingivitis and periodontal disease were 90%, 98% and 90.33% respectively [9a]. Among the cases with periodontal disease bleeding on probing was the commonest finding (62.5%), supragingival and subgingival calculus (22.6%), and pockets (14.9%). 68.60% of cases were given oral hygiene instruction only (twice daily brushing with fluoride paste). 18.97% of cases were given oral amoxicillin, paracetamol and advise regarding oral hygiene.

43% of cases required scaling, plaque removal, oral antibiotic and oral hygiene. The procedure was timed in the second trimester. The cases were subsequently followed up in second and third trimester [9a]. There was a significant improvement in the oral health in the second and third trimester in the treated group compared to the untreated group. The OHI-S index showed perfect oral hygiene in 0%, good oral hygiene in 15.24%, satisfactory hygiene in 56.64% and bad oral hygiene in 28.12% of cases. The OHI-S index <1 was considered good and ≥1 was considered bad. Poor hygiene and illiteracy/low education status was significant predictor of dental caries and gingivitis. Those cases with poor oral hygiene were 20 times more likely to have gingivitis than those practicing good hygiene. Odds ratio220.00, 95%CI (27.4286-1764.5812) Z statistic 5.077,P-value<0.0001. The pregnant women with poor dental care were 2.5 times more at risk of caries than those practicing good dental care (Odds ratio 72.6883, 95% CI (17.5356-301.3063), Z statistic 5.908, P-value <0.0001) [9a].

Table 1. Dental diseases in pregnant women

Dental disease	Pregnant women Number (total cases 600)	Percentage %
Caries		
Yes	540	90%
No	60	10%
Gingivitis		
Yes	588	98%
No	12	2%

Table 2. Association of dental caries and oral hygiene status in pregnant women

Oral hygiene status	Caries number percentage	No caries number percentage
Good	154 28.5%	58 96.7%
Poor	386 71.5%	2 3.3%

OR=72.6883, 95%CI (17.5356-301.3063)
Z statistic 5.908, P-value <0.0001

Table 3. Association of gingivitis and oral hygiene status in pregnant women

Oral hygiene status	Gingivitis number percentage	No gingivitis number percentage
Good	28 4.8%	11 91.7%
Poor	560 95.2%	1 8.3%

OR=220.00, 95% CI (27.4286-1764.5812)
Z-statistics 5.077, P-value <0.0001

4. DISCUSSION

Pregnancy is a state when women can be motivated to adapt healthy behaviour. Dental and obstetric teams can work as team to inculcate life long oral hygiene habits to oneself and to the family. The ideal number of dental checkup is two in 1st trimester and one in second and third trimester. When medication is needed penicillin, erythromycin and cephalosporins are safe. Pain originating from the teeth can cause contractions, hence analgesics may be advised. Acetaminophen is preferred drug throughout pregnancy for pain [9,10].

The increase in estrogen and progesterone secretion in pregnancy causes gingivitis, gingival hyperplasia, pyogenic granuloma, salivary changes and dental caries [9a]. The effect is first noticeable in the second month of gestation and peaks in eight month. Some recent studies have shown the prevalence of gingivitis 86.2-98.8%. The prevalence of dental caries 74%. The frequency of pyogenic granuloma varies from 0-9.6% [1,2]. In the study, it was found the prevalence of dental caries, gingivitis and periodontal disease were 90%, 98% and 90.33% respectively. The high prevalence of dental disease can be attributed to poor hygiene, low literacy and low socioeconomic status in the study group. Studies have shown a higher prevalence of gingivitis and dental caries among pregnant women with poor oral hygiene and low education status [7,8]. In our study, the cases with poor oral hygiene were 2.5 times more likely to have dental caries and 20 times more likely to have gingivitis. The periodontal disease has been associated with increased risk of preterm birth, low birth weight, preeclampsia and intrauterine growth retardation. The periodontal disease represents an infectious disease affecting more than 23% of women between the age group 30-54 years [11, 9a].

The dental management guidelines during pregnancy: Pregnant women should be considered as a prime target group for oral health education [12,13].

First trimester (1-12 wk):

- Dental checkup and assessment of current dental health status.
- Provide health information and counselling.
- Educate regarding oral hygiene and preventive care.
- Avoid procedures
- Avoid routine radiographs.

Second and Third trimester:

- Risk of vena caval syndrome, avoid prolong sitting in dental chairs and prefer left side position and elevate head of chair.
- Scaling, polishing, curettage can be done in second trimester if necessary.
- Oral hygiene to be followed.
- Avoid radiographs.
- Control active oral infections.

5. CONCLUSION

It is strongly recommended that dental examination by a dentist/trained medical staff of all the antenatal cases should be mandatory in outpatient department of maternity hospital. A dental examination and appropriate dental care should become integral part of the routine management of every pregnant woman and that of the medical curriculum for medical students. The preventive and primary care should be management goal for dental health in pregnancy. The clinician should educate the women of childbearing age to seek oral health counselling and examination in preconception period and as soon as pregnancy is confirmed. Implementing preventive dentistry at primary level will improve prenatal care and outcome.

ETHICAL APPROVAL

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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I completed my MBBS, MD, FICOG, CIMP, and FCSM and now working as a Professor and Head at Dr KN Singh Memorial Institute of Medical Sciences in the department of Obstetrics and Gynaecology. I have published around 30 articles in National and International journals and have reviewed journals as a Reviewer. My field of interests mainly include sexual medicine and menopausal health. I supervised research as a Guide and co-guide for MD OBG, PhD, D Pharma, Medical Microbiology, and Medical Anatomy students. I believe that learning brings motivation and innovation to evolve as a person.

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Determination of Interfraction Variation in Interstitial High dose Rate Brachytherapy

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ABSTRACT

The positional stability of the catheters and the resultant dosimetric variation over a period of time is studied and presented. The ability of brachytherapy to deliver high radiation doses over a short time period means patients can complete treatment in days rather than weeks required for EBRT. Brachytherapy is generally well tolerated with a good toxicity profile for many of its applications, largely due to its tissue sparing approach. The remote after-loading HDR Brachytherapy treatment unit Gamma Med iX plus (Varian Medical Systems, Palo Alto, CA) or Microselectron HDRV3 (Nucletron, BV) using single sealed Iridium 192 radioactive source was used for treatment and for treatment planning, Eclipse (Varian Medical Systems, Palo Alto, CA) or Oncentra Master plan (Nucletron, BV) was used. Interfraction errors occur frequently in interstitial HDR brachytherapy. If no action is taken it will result in a significant risk of geometrical miss and overdose to the organs at risk. The effect of interfraction variation also depends on the fractionation schedule of the brachytherapy treatment. It is recommended to complete the treatment within 5 days. For cancer cervix (MUPIT) interstitial implant planning before each fraction is recommended.

Keywords: Microselectron; cancer cervix; brachytherapy treatment; cancer management.

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1. BACKGROUND

It is estimated that there will be 22.2 million new cases diagnosed with cancer by 2030 worldwide [1]. The worldwide incidence of squamous cell carcinoma of head and neck is more than 500,000 cases per year and the management of patients with head and neck cancer are complex [2]. Cancer management generally has undergone major advances since the 1960s and there have been notable improvements over time in the probability of survival from most of the common cancer types. The choice of treatment modality depends on the stage and site of disease. Radiation plays a dominant role in treatment of cancer. Radiation therapy is sometimes given in a combination of brachytherapy and external beam therapy. Main advantage of brachytherapy is that it delivers high radiation dose to tumor while optimally sparing the surrounding normal tissue. Brachytherapy plays an integral role in the management of cancers and has been described as the first form of conformal radiation [3]. Precise source placement enables very high doses within the tumor and sufficient dose at the margin between the tumor and normal tissue ensuring high tumor control. At the same time, only small volumes of normal tissue is irradiated thus decreasing the complications. Interstitial Brachytherapy involves the implantation of plastic or metal needle catheters (applicators) into tumors.

Time-tested Low Dose Rate (LDR) brachytherapy was gold standard in brachytherapy till 1980's. After the invention of High Dose Rate (HDR) brachytherapy (BT), low dose rate brachytherapy is losing sheen, due to its undue disadvantage like low dose rate where patients have to be monitored continuously for 2 to 3 days and more radiation exposures to the oncologist and support staff. While High dose rate brachytherapy has high dose rate and patients can be treated on an outpatient basis with less radiation exposure to oncologist and support staff. Short treatment time leads to less patient discomfort, and complications of prolonged bed rest are eliminated. Integrations of external beam radiation therapy (EBRT) and brachytherapy are possible with HDR, thus reducing overall duration of treatment and potentially a better tumor control. The ability of brachytherapy to deliver high radiation doses over a short time period means patients can complete treatment in days rather than weeks required for EBRT. Brachytherapy is generally well tolerated with a good toxicity profile for many of its applications, largely due to its tissue sparing approach.

Traditionally treatment planning of brachytherapy was mainly based on radiographs and point dosimetry [4, 4a]. The dose distribution was related to the geometry of the catheters. With the newer three-dimensional treatment planning systems together with Computer Tomography (CT) imaging, it is possible to get a 3D based dose distribution with reconstruction of the tumor volume and the catheters [5]. Advanced computerized treatment planning and image-guided delivery systems increase efficiencies and improve outcomes. It achieves this through the placement of a radioactive source within or adjacent to a tumour using specially designed applicators and remote, computer-controlled delivery devices [4a]. This allows a tailored radiation dose to be delivered very precisely to the target area. The use of imaging techniques, such as ultrasound, CT,

Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) for treatment planning, has led to improved visualization of the tumor and surrounding organs. The use of multiple imaging techniques can help and improve the treatment delivery process, and allow real-time changes to dose and applicator positioning [4a].

Although brachytherapy continues to be a key cornerstone of cancer care, it is clear that treatment innovations are needed to build on this success and ensure that brachytherapy continues to provide quality care for patients [4a]. The dosimetric advantages offered by High Dose Rate brachytherapy to the tumor volume rely on catheter positions being accurately reproduced for all fractions of treatment. However, catheter migration is often observed between fractions [6,7]. This leads to a significant risk of under dosage to the tumour or over dosage to the organs at risk. Correction of catheter migration becomes more important. Catheter position can change during treatment or between fraction resulting in shifts of source dwell positions relative to the target structures and organs at risk, and thereby changing the delivered dose.

The positional stability of the catheters and the resultant dosimetric variation over a period of time is studied and presented [4a].

2. MATERIALS AND METHODS

The remote after-loading HDR Brachytherapy treatment unit GammaMed iX plus (Varian Medical Systems, Palo Alto, CA) or Microselectron HDRV3 (Nucletron, BV) using single sealed Iridium 192 radioactive source was used for treatment and for treatment planning, Eclipse (Varian Medical Systems, Palo Alto, CA) or Oncentra Master plan (Nucletron, BV) was used. Images for planning were acquired by CT Somatom spirit (Siemens) two slice. Sixty-six patients were included in this study over a period of 22 months from December 2011 to September 2013 [4a]. Patient's demography is given in Table 1. The patients were treated after evaluation according to the stage of the disease as per the institute treatment protocol given in Table 2.

Table 1. Patient characteristics [4a]

Characteristics	No of patients (%)
Age	
Mean	49.98
Standard Deviation	9.82
Median	49
Range	32-73
Gender	
Male	31 (46.97%)
Female	35 (53.03%)
Diagnosis	
Carcinoma Breast	14 (21.21%)
Carcinoma Buccal Mucosa	21 (31.82%)

Characteristics	**No of patients (%)**
Carcinoma Cervix	3 (4.54%)
Carcinoma Floor of Mouth	2 (3.03%)
Carcinoma Tongue	21 (31.82%)
Soft tissue sarcoma (multiple site)	3 (4.55%)
Carcinoma Lip	2 (3.03%)
T stage*	
T1	6 (9.09%)
T2	25 (37.88%)
T3	35 (53.03%)
T4	0 (0%)
N stage*	
N0	48 (72.72%)
N1	13 (19.70%)
N2	5 (7.58%)
N3	0

** According to the 7th American Joint Commission on Cancer/Union for International Cancer Control Staging system*

Table 2. Institutional Treatment protocol [4a]

Diagnosis	**EBRT***	**HDR ****
Carcinoma Breast	200 c Gy x 20 fractions	250 c Gy x 6 fractions
Carcinoma Buccal Mucosa	200 c Gy x 25 fractions***	350 c Gy x 6 fractions
Carcinoma Cervix	200 c Gy x 25 fractions	400 c Gy x 5 fractions
Carcinoma Floor of Mouth	200 c Gy x 25 fractions ***	350 c Gy x 6 fractions
Soft tissue sarcoma	200 c Gy x 20 fractions	250 c Gy x 6 fractions
Carcinoma Tongue	200 c Gy x 25 fractions ***	350 c Gy x 6 fractions

** Five fractions per week with one fraction per day.*
*** Two fractions per day with 6 hours gap between the two fractions.*
**** With Spinal shield after 44 Gy.*

2.1 Patient Evaluation

Patinet history, physical examination, diagnostic studies and reports were evaluated. The extent of the disease are estimated and recorded with staging, nodal involvment and metastasis. The clinical target volume is determined and recorded.

2.2 Interstitial Implant Application

Under general anaesthesia, trocars and hollow needles were inserted as guide tubes in and around the tumor 1 cm apart in single or multiple planes through which plastic tubes were threaded. Theses tubes were then secured by buttons. Patient with flexible catheter implant is shown (Fig. 1). Similarly, for rigid needle implant, the sterilized needles with the appropriate length were selected [4a].

With the guidance of templates, the needles were inserted into the tissue. The template helped to maintain proper geometry of the needle placement. The needles were secured by stainless steel buttons (Fig. 2). Table 3 gives the characteristics of the interstitial implants. The oncologist takes the responsibility of inserting and removal of the catheters and the procedure is carried out in minor operation theratre (OT) [4a].

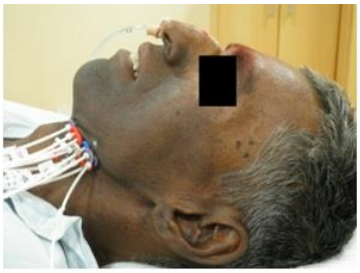

Fig. 1. Patient with flexible interstitial implant [4a]

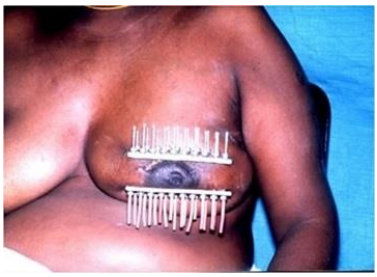

Fig. 2. Patient with rigid needle implant [4a]

Table 3. Implant Characteristics [4a]

Type	**No of patients (%)**
Rigid Needle Implants	17 (25.76%)
Flexible Catheter Implants	49 (74.24%)
Number of Planes	
Single Plane	21 (31.82%)
Double Plane	39 (59.09%)
Multiple Plane	6 (9.09%)

2.3 Imaging and Planning

On the second day after implantation, the patients underwent CT scan of the involved region with a slice thickness of 1mm. Minimum 5 cm of margin from target is given superiorly and inferiorly while imaging. Whereas for cancer cervix Martinez universal Perineal Interstitial Template (MUPIT) patients, CT imaging

was done immediately after implanting the catheters and treatment were delivered within 1-2 hours. Medical Physicists along with oncologist are involved during treatment planning. The delineation of target and organs at risk are drawn on the images by oncologist. Medical Physicist does catheter reconstruction, source placement, normalization and optimization of the dwell time. On the CT images, the applicator reconstruction was done, and at the tip of all the applicators a reference point was inserted. The reconstructed catheters in Treatment Planning System (TPS) with reference points are shown (Fig. 3). These reference point act as the tracking tool to monitor the catheter movement between both the plans. The source dwell positions and step size were identified, and accordingly the fine-tuning of dose optimization was performed by changing the dwell time and weighted for individual dwell positions. In most cases, dwell time was changed to reduce the hot spot or to remove the cold spot. Graphical optimization was never used. It was ensured that at least 90% of the clinical target volume receives the prescribed dose. The dose distribution was generated by TPS using the AAPM TG-43 dose formalism [8, 4a]. Treatment was delivered using the HDR remote after-loading system. On the last fraction, a repeat CT (Post HDR) and replanning was done, and the catheters were removed.

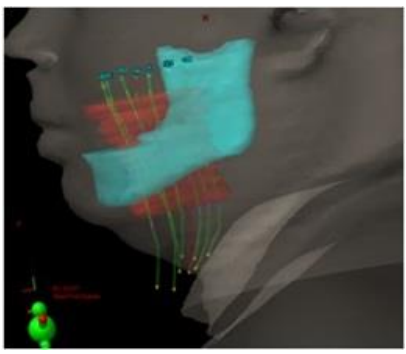

Fig. 3. Reconstructed implant with a reference point [4a]

For each patient, planning was done on both Pre HDR and Post HDR images. Pre HDR and Post HDR images were fused based on, a) the prominent anatomical landmarks close to the clinical target volume, b) anatomical landmarks that have no positional variation, c) catheter geometry and d) template positions [4a].

The step size dwell position and dwell time was maintained the same in both the plans and only the catheter position was updated in the post HDR brachytherapy plan. The tip of the catheters where the reference points were inserted gave the co-ordinates in x, y and z-axis. The variation in the reference points between the two plans was estimated, which gave the actual displacement in, the catheter position in 3D axis [4a]. The schematic representation of plan fusion and the reference point analysis is shown (Fig. 4). Using the DVH the dosimetric

parameters were studied for both the plans and the dosimetric variation was estimated.

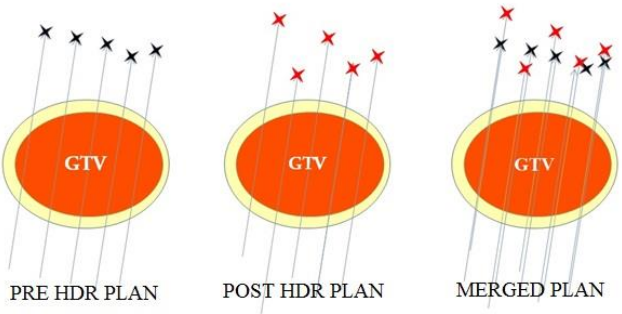

Fig. 4. Schematic diagram of plan fusion [4a]

2.4 Treatment Delivery

Patient identities are verified. The treatment plans were transferred from the treatment planning system to HDR treatment unit. Qualified radiotherapy technologists were involved in treatment planning. The appropriate channels are to be connected to the catheters and it is done under the supervision of radiation oncologist and medical physicists. The treatment time in the treatment unit are cross-verified with the plans followed by the treatment delivery. The delivered treatment is documented.

2.5 Plan Analysis

The variation in the reference point (3D vector) between the two plans was obtained. The geometrical displacement of the catheters is thus estimated. If there is no displacement of the catheters it is expected to have the same co-ordinate values in both the plans which will result in co-ordinate X=0, Y=0 and Z=0. Any variation or movement in the catheter will have some definite values. The dosimetric variation for all the reference points was also obtained [4a]. To estimate the volumetric data, the dose received by 90% of clinical target volume (D_{90}) was obtained from the DVH. The other parameters that were obtained were volume receiving more than 150% of the given dose $V_{150\%}$.

3. RESULTS

For fourteen patients with carcinoma breast the displacement in catheter position and dosimetric variation are shown (Fig. 5 & Fig. 6). For all the patients, the catheter displacement and D_{90} dose to clinical target volume were less than 3mm and 3% respectively [4a].

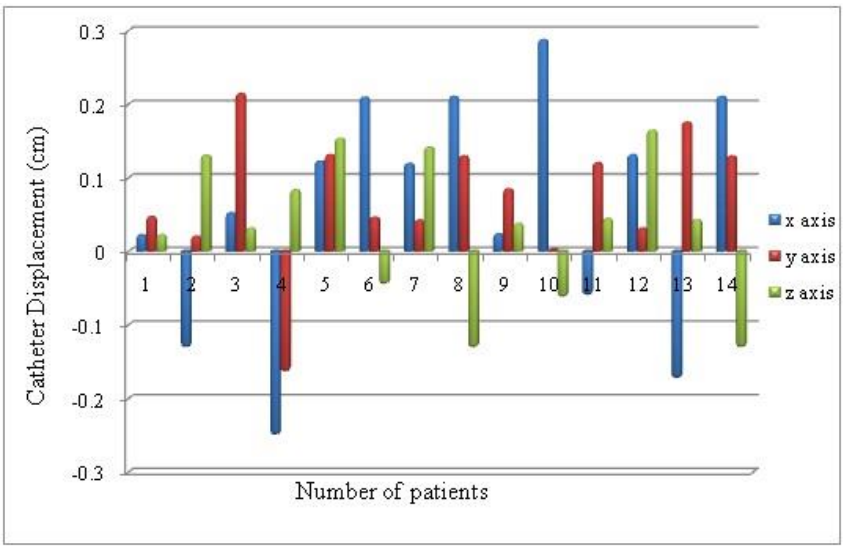

Fig. 5. Catheter displacements for Carcinoma Breast [4a]

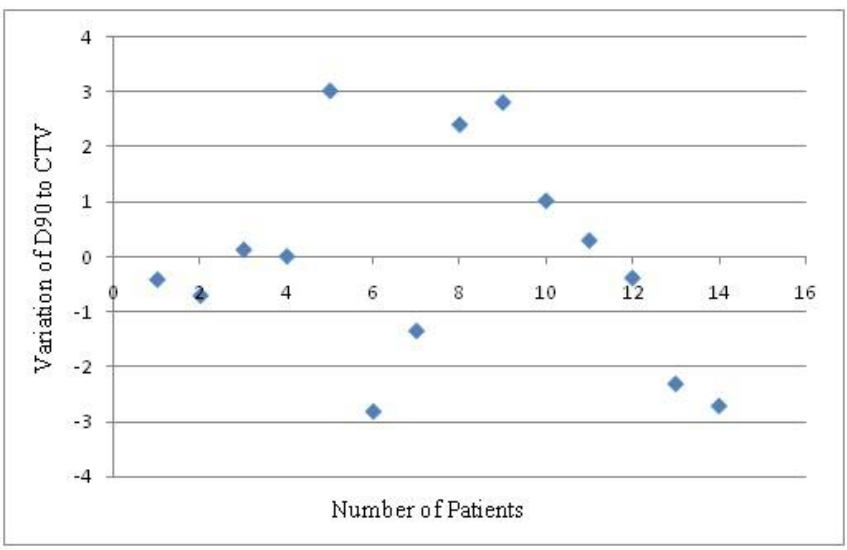

Fig. 6. Dosimetric variations for Carcinoma Breast [4a]

For twenty one patients with carcinoma buccal mucosa, the catheter displacement for 33.33% of the patients was more than 5mm (Figs. 7 & 8). In 38.10% of the patients, D90 dose to clinical target volume was more than 3% (Fig. 9).

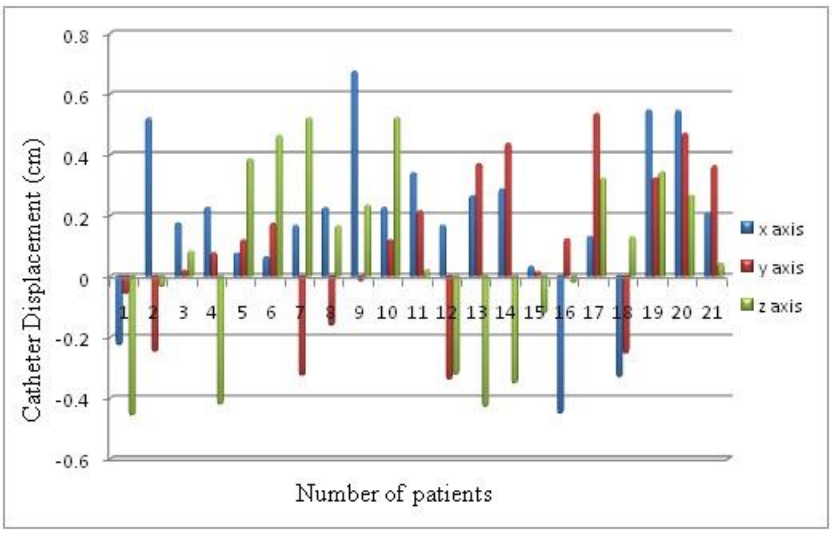

Fig. 7. Catheter displacements for Carcinoma Buccal mucosa [4a]

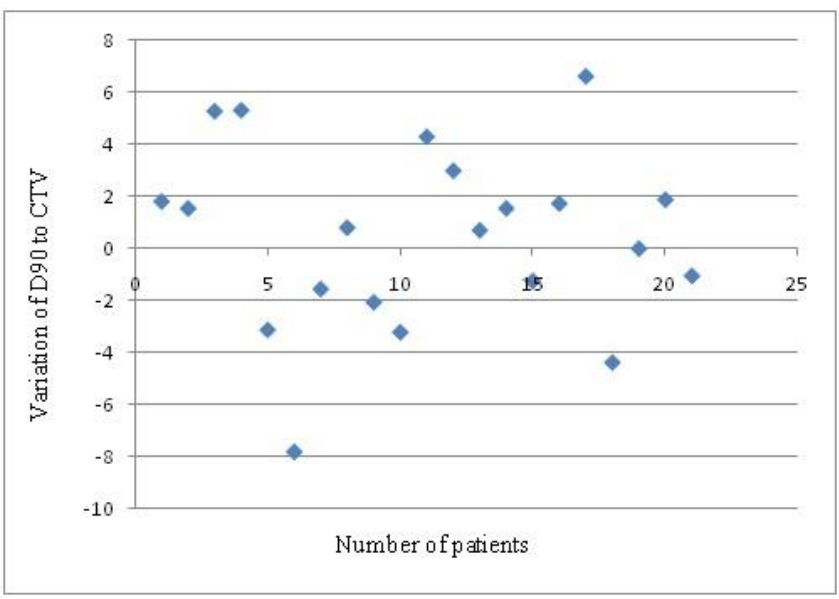

Fig. 8. Dosimetric variations for Carcinoma Buccal mucosa [4a]

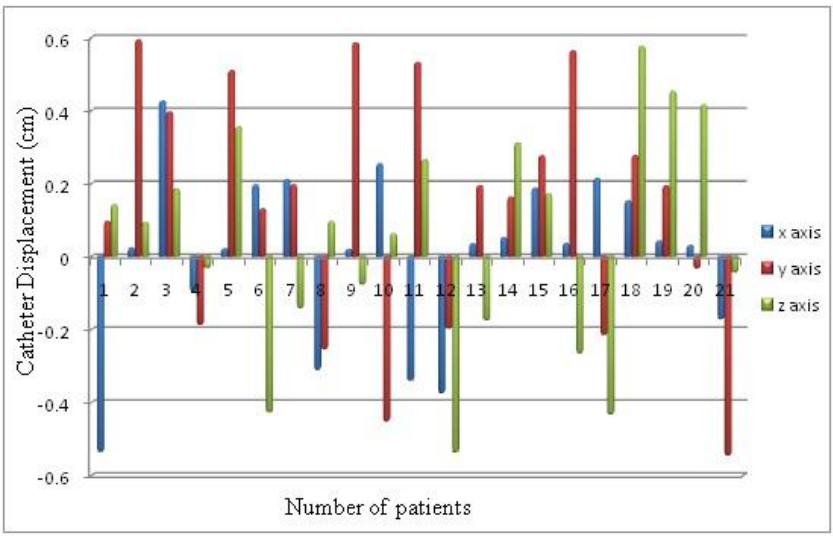

Fig. 9. Catheter displacements for Carcinoma Tongue [4a]

For twenty one patients with carcinoma tongue, the displacement in catheter position are shown (Fig. 9). The catheter displacement for 38.10% of patients was more than 5mm. As per DVH data in 28.57% of the patients D_{90} dose to clinical target volume was more than 3% (Fig. 10).

Fig. 10. Dosimetric variations for Carcinoma Tongue [4a]

The catheter displacement for ten patients with carcinoma cervix (3 patient), carcinoma floor of mouth (2 patients), soft tissue sarcoma (3 patients), and carcinoma lip (2 patients) are shown (Fig. 11). The dosimetric variations are shown (Fig. 12).

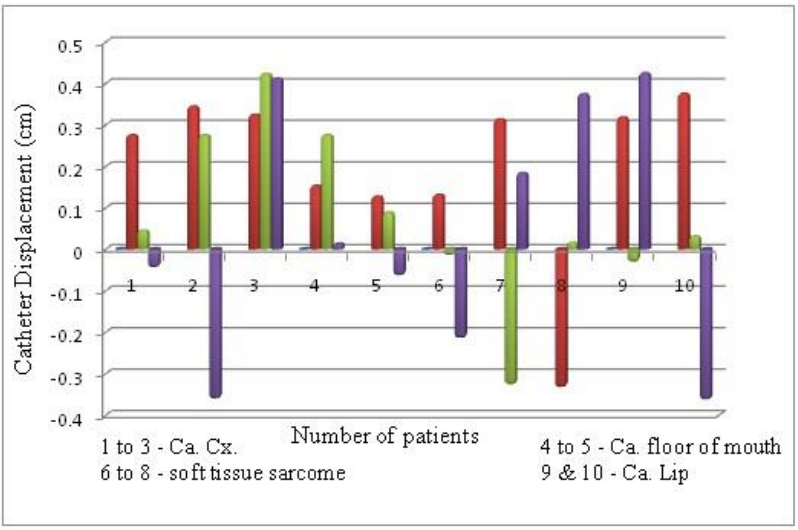

Fig. 11. Catheter displacements for other sites [4a]

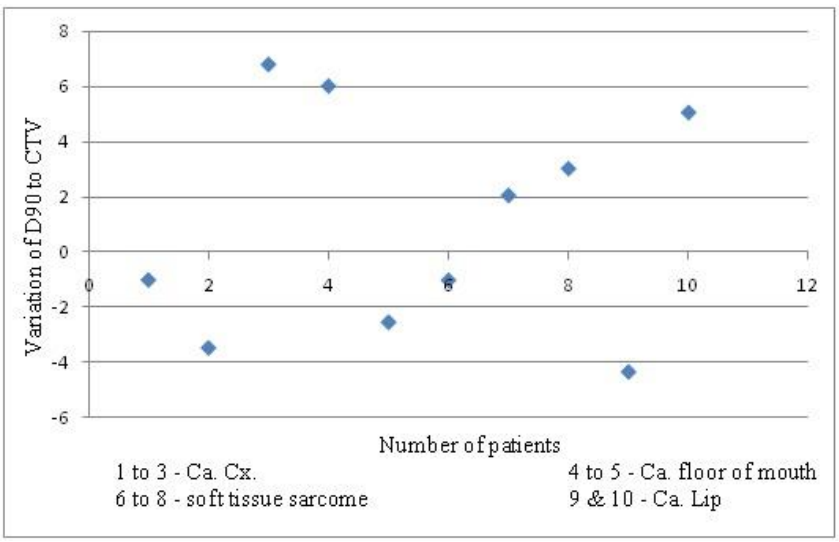

Fig. 12. Dosimetric variations for other sites [4a]

Table 4 gives the details of the mean dose variation in percentage to D_{90} of clinical target volume.

Table 4. Dosimetric variation in percentage to D_{90} of CTV [4a]

Type of Tumor	Mean Value + 1 Standard Deviation
Carcinoma Breast	1.45 ± 1.16 (14 patients)
Carcinoma Buccal Mucosa	2.81 ± 2.09 (21 patients)
Carcinoma Tongue	2.25 ± 1.17 (21 patients)
Soft Tissue Sarcoma	2.52 ± 1.28 (03 patients)
Carcinoma Floor of Mouth	4.60 ± 5.37 (02 patients)
Carcinoma Cervix (MUPIT)	3.76 ± 2.89 (03 patients)
Ca. Lip	4.70 ± 0.49 (02 patients)
D_{90} - Dose received by at least 90% of the volume.	

The dosimetric variation to volume receiving more than 150% of the prescribed dose are given in Table 5.

Table 5. Dosimetric variation to volume receiving 150% of dose [4a]

Type of Tumor	Variation in Volume receiving more than 150% of dose	Type of Tumor
Carcinoma Breast	1.8 cm^3	(1.76%)
Carcinoma Buccal Mucosa	6.42 cm^3	(2.14%)
Carcinoma Tongue	1.04 cm^3	(7.91%)
Soft Tissue Sarcoma	1.3 cm^3	(2.31%)
Carcinoma Floor of Mouth	2.8 cm^3	(2.85%)
Carcinoma Cervix (MUPIT)	1.2 cm^3	(1.35%)

4. DISCUSSION

A preliminary analysis with 55 patients was done and concluded that with increase in the treatment duration an increases in inter-fraction error occurs [9]. Inclusion of carcinoma lip patients in this study gave some new findings that edema was also a cause for inter-fraction error and needs to be reported. N Naiyanet et al. [10] on patient's setup variation in EBRT has concluded that the population-based margin was less than 5 mm; thus the margin provides sufficient coverage for all of the patients. The results suggest that the margin given in EBRT from clinical target volume to planning target volume is adequate and improves the confidence in patient specific margins. Whereas, "in HDR brachytherapy similar margin to clinical target volume" is not given which takes into account the positional variation of the catheters [4a].

Thanigaimalai Velmurugan et al. [11] studied the dosimetric variation due to interfraction organ movement in HDR interstitial (MUPIT) brachytherapy for gynecologic malignancies on ten patients. They estimated the variation in the volume of clinical target volume. In one of the ten patient studied, there was an

increase of clinical target volume which has increased by 1.04% and the maximum decrease in volume was 6.9% [4a]. The reduction in volume is because of decrease in edema. The average volume variation was found to be -3.4%. The mean dose to clinical target volume variation was 9.8% to -13.3%. Similarly, the bladder volume variation was in the range of +28.6% to -34.3% and for rectum 38.4% to -14.9%. The range of mean dose variation to bladder was +17.1% to -66.2% and to rectum it was 14.0% to -0.8% [4a]. They have concluded that the volumetric changes seen in bladder, rectum and clinical target volume are patient specific, and no correlation was seen with volumetric changes to dose. They had suggested for re-planning before each treatment was delivered.

Kailash Narayan et al. [12] in an expert review article has discussed the advantage of image guided brachytherapy and strongly recommended that image-guided brachytherapy should be considered as standard of care for treatment in brachytherapy. In our study, We have identified that image guidance in fractionated brachytherapy will reduce the interfraction error considerably [4a].

Nicole Nesvacil et al. [13] evaluated a comparison of the dosimetric impact of inter and intrafraction anatomical variations in fractionated cervix cancer brachytherapy and reported relative systematic and random changes of D_{90} of High Risk clinical target volume. Kirisits et al. [14] and Mohamed et al. [15] pointed out that replanning of individual fractions is advisable for consecutive applicator insertions when interstitial needles are used. They have also pointed out that limited data was available in their sample, and no significant differences of dosimetric variations for different implant types were detected [4a].

A review of relevant literature revealed that very limited study was carried out to estimate the interfraction error in interstitial HDR brachytherapy corresponding to various sites of cancer [4a]. In our study, we have tried to evaluate the positional variation of the catheters and noticed that, a correlation occur between the positional variations of catheters and dose. We have given the positional variation of catheters in 3D vector and the dosimetric variation to clinical target volume (D_{90}, V_{150}). Our study also reveals that, suturing of the buttons with the skin does not provide a solution to prevent catheter displacement. Suturing restricts button movement whereas the flexible catheter made of nylon slides through the buttons. Fig. 13 shows button displacement in a flexible nylon catheter interstitial implant. To control the physical movement of catheters in flexible catheter implants, plaster of micro-pore flags are fixed close to the distal end of the buttons, this helps in preventing the buttons getting dislodged from its position (Fig. 14). This method of using the micro-pore plaster not only helps to identify the catheter to be connected to the afterloader but also prevents the geometric movement of the catheters [4a].

In our study, we have analyzed the interfraction error with respect to various sites. It was found that the variation was more for cervix patients with MUPIT implants [4a]. For cancer lip patients, edema is the major issue for variation. Hence, for cancer lip patients it is recommended to give a waiting period of 2-3

days, for edema to subside, followed by imaging and planning. For cancer tongue patients, the mobility of the tongue results in catheter displacement [4a].

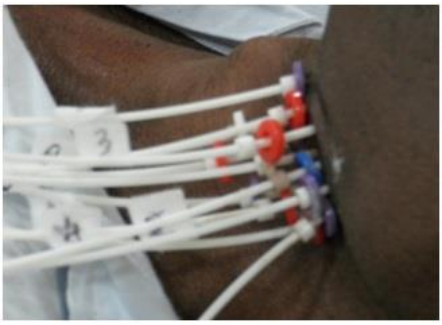

Fig. 13. Displaced buttons in flexible catheter interstitial implant [4a]

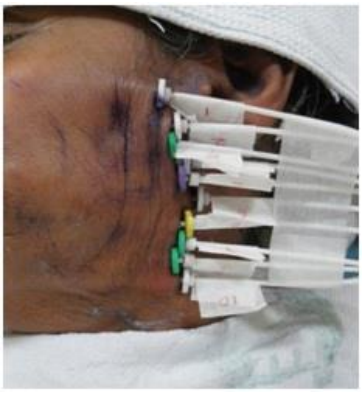

Fig. 14. Micro pore plaster preventing the buttons displacement [4a]

Positional errors associated with the physical insertion of catheters with transfer tube are also identified [4a]. Those authors that have described implant verification [16] using fluoroscopy or radiographs relative to bone structures have demonstrated that there are also soft tissue changes, which can affect implant geometry.

As per AAPM Report 41 [17] the dosimetric variation should be limited to 3%. As per our results it has been identified that for carcinoma breast the interfraction variation was least. The same rigid needle implant used for treating carcinoma cervix gave different results [4a].

For carcinoma floor of mouth and carcinoma lip, the dosimetric variation was more.

The AAPM task group 303 [18] recommends that the treatment setup verification is an integral part of the procedure workflow. To ascertain the treatment geometry conforms to the planned geometry, pretreatment imaging is recommended for each treatment fraction for a multifraction, single implant workflow or when there is a substantial delay between planning simulation and treatment. This will also require the establishment of action thresholds and remediation procedures in case of a discrepancy is observed. To do so efficiently, it is recommended that fiducial markers implanted in the target volume be used as references to assess applicator or catheter displacement. If fiducial markers are used, a physician/oncologist should place them prior to the planning simulation. Bony references and other anatomical surrogates (such as Foley balloons) are not recommended, as they do not provide information on the position of the applicator with respect to the target.

5. CONCLUSION

Interfraction errors occur frequently in interstitial HDR brachytherapy [4a]. If no action is taken it will result in a significant risk of geometrical miss and overdose to the organs at risk. In contrast to brachytherapy, these effects have been studied extensively in the field of EBRT for more than 20 years [19]. The findings of this study justify additional imaging between fractions in order to make a decision for replanning is necessary. Introduction of image-guided brachytherapy becomes more essential, this will not only assist applicator placement but also helps to assess the applicator displacement between fractionated brachytherapy. Hence Image-guided brachytherapy should be standardized as done in EBRT. It is recommended to do imaging before each fraction and replanning are recommended if the geometrical variation of applicators is more than 5mm [4a]. The effect of interfraction variation also depends on the fractionation schedule of the brachytherapy treatment. It is recommended to complete the treatment within 5 days. For cancer cervix (MUPIT) interstitial implant planning before each fraction is recommended.

For cancer lip and tongue post-implant edema are the main cause for catheter displacement. It is recommended to give 2 to 3 days interval after implants are done and then to do the imaging and planning. For all flexible catheter implants in addition to the buttons the micro pore plaster flags are recommended which not only help to restrict the displacement of catheters but also helps to identify the catheter number to be connected to afterloader [4a].

Overall it is strongly recommended to image before each fraction and compare with the planned image. Based on the catheter position judgments are to be made for correction of interfraction catheter movement. The upcoming trend of MRI imaging over CT image are increasing. According to the 2014 American Brachytherapy Society (ABS) practice pattern [20] survey, the utilization of MRI in GYN HDR BT has increased. Additionally, within this timeframe, an increase in volume based target delineation (high-risk CTV) from 14 to 52% was observed. Recommendations for MRI and volume based treatment planning have largely been based on the guidance GEC ESTRO [21-24].

However, there is considerable variation from patient to patient; some patients seeing little change between all fractions, with or without catheter displacement. The limitations of our study are the use of DVH as a representative parameter. It will be more appropriate to perform in-vivo dosimetry with MOSFET or TL material, which gives more relevant dosimetric data, this will help to intervene and revise the plan [4a]. As immobilization devices are not used in brachytherapy, reproducibility during imaging becomes difficult. This results in uncertainties during fusion of the pre HDR and post HDR plans.

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 7
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Understanding the Pathoanatomy of Arthritic Hip for Improvising the Technique in Primary Uncemented Total Hip Arthroplasty

Paresh Golwala [a++*], Parth N. Rathi [b#] and Prassana Shah [c†]

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ABSTRACT

Background: Hip osteoarthritis is a condition that leads to significant morbidity and decreases the quality of patient's life. Total Hip Arthroplasty (THA) is preferred by most of orthopaedic surgeons to relieve pain, provide motion while maintaining stability. In this study we aim to discuss the difficulties encountered in reference to patho-anatomy during THA in various conditions along with their solutions.
Materials and Methods: In our study, we had observed 100 hip joints of secondary osteoarthritis in 67 patients of with different type of underlying pathology like avascular necrosis, sickle cell disease, rheumatoid arthritis, ankylosing spondylitis. All the patients were evaluated using modified harris hip score. The findings were evaluated using SPSS software (Ilionois, Chicago) with p value less than 0.05 considered as significant.
Observation and Results: We had operated 100 hip joints (n=100) having arthritic changes among 67 patients (33 patients with bilateral hip affection, 34 patients with unilateral hip affection) at our tertiary care hospital from June 2015 till December 2019. The mean Harris Hip Score at the final follow up was 88 among them 98 hip joints had excellent and 2 hip joints had good to fair results. During our study we majorly encountered three types of collapse – Anterosuperior collapse (56%), Concentric collapse (25%) and hip arthritis due to inflammatory conditions (19%). We had found that according to duration and

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types of collapse different soft tissues were contracted and a step-wise release of those structures is necessary for proper intra-operation reduction and post-operative better range of motion. The steps for global release as and when required in different patients include adductor tenotomy, release of external rotator muscles, release of anterior fibers of gluteus medius, release of tensor fascia lata and tendinous insertion of gluteus maximus along with release of posterior capsule, release of anterior capsule followed by release of iliopsoas tendinous insertion on lesser trochanter.

Conclusion: The chronicity of the disease will lead to anatomical changes of the surrounding soft tissue and that will affect the surgical dissection. We had described surgical problems encountered during the operation and their solutions for each type of pathoanatomy. This will help orthopedic surgeons to anticipate and prepare well in advance while performing total hip arthroplasty.

Keywords: Pathoanatomy; hip arthroplasty; hip arthritis; AVN; sickle cell disease; contracture release.

1. INTRODUCTION

Osteoarthrosis is a progressive degenerative joint disease that causes damage to the articular cartilage and surrounding structures. Osteoarthritis occurs when further degeneration of cartilage leads to rubbing of two bones with open nerve endings against each other [1-5]. This leads to pain and protective muscle spasm. Spastic muscle over a very long period of time loses its elasticity leading to fibrosis and contracture. Furthermore, capsules, ligaments and surrounding soft tissues undergo permanent contracture and shortening. This leads to painful and deformed joint called osteoarthritis. The hip is the second most commonly affected degenerative joint (after the knee), affecting millions of people worldwide. Hip arthritis is a condition that leads to significant morbidity and decreases the quality of life [6-9]. Symptoms of osteoarthritis can usually be effectively managed conservatively in early stages, although the underlying process cannot be reversed thus increasing the need for a surgical procedure like total hip arthroplasty (THA) at an early age [10-13]. Total Hip Arthroplasty is preferred by most of orthopaedic surgeons to relieve pain, provide motion while maintaining stability and to correct the deformity as it provides a painless, mobile and stable joint [14-19]. The incidence of Arthroplasty has increased over the past decade and expected to increase more in future. Total hip arthroplasty can be either cemented / uncemented or hybrid [20-26]. The success of THA in older patients, in concert with improvement in techniques and biomaterials, has stimulated demand for this procedure in younger, more active patients hoping to regain full activity [27-30]. Operating steps for THA has been standardized, however patients with different underlying presentations may have different patho-anatomy of hip and surrounding soft tissues. In this study we aim to discuss the difficulties encountered related to soft tissue contractures during THA in various conditions along with their solutions.

2. MATERIALS AND METHODS

The study was conducted at tertiary care centre from June 2015 to December 2019 after getting ethics committee approval (SVIEC

/ON/MEDI/BNPG/15/D16207). In our study, we had observed secondary osteoarthritis of hip with different types of underlying pathology like avascular necrosis, sickle cell disease, rheumatoid arthritis, ankylosing spondylitis. All patients who came to OPD with suspected case of hip arthritis were primarily examined and deformity with restriction of movement were noted. This was followed by getting standard radiographs of pelvis with both hips anteroposterior views and thereafter frog-leg views (abduction and external rotation). Also, MRI (Magnetic Resonance Imaging) was done for all our patients for pre-operative evaluation. On the basis of clinical findings and radiological investigations, preoperative planning was done based on templating. We had done all THA (Total hip Arthroplasty) with Modified Gibson Approach. The patients were encouraged to walk with the help of walker under the supervision of physiotherapists. The patients were sent home within one week of operation. They were subsequently followed at outpatient clinics at second week, six weeks, ten weeks and twenty-four weeks. All the patients were evaluated using modified Harris hip score. The findings were evaluated using SPSS software (Ilionois, Chicago) with p value less than 0.05 considered as significant.

3. RESULTS

We had operated 100 hip joints (n=100) having arthritic changes among 67 patients (46 male patients and 21 female patients). 33 patients had bilateral hip affection (20 male patients and 13 female patients) and 34 patients had unilateral hip affection (26 male patients and 08 female patients). All patients were operated and followed up at our tertiary care hospital from June 2015 till December 2019. The mean age of the patients was 36±8.5 years (34±5.6 years in males and 37±2.7 years in females). The common causes of avascular necrosis of femoral head include alcoholism (n=49), sickle cell disease (n=25), inflammatory disorders (n=19) and steroid intake (n=7). We performed more uncemented total hip arthroplasties (n=87) as compared to hybrid total hip arthroplasty (n=9) and cemented total hip arthroplasty (n=4). The patients were followed up for 28.7±13.3 months with longest follow-up of 42 months. The Harris Hip Score at the final follow up was excellent for 98 operated hip joints and good to fair in 2 hip joints.

3.1 Observation

We encountered patients for THA with osteoarthritis due to various causes with variable deformities and limb length discrepancy. As per our observations, there are three different types of arthritic hip presentations. This includes anterosuperior collapse of femoral head following avascular necrosis (56%), concentric/global collapse of femoral head (25%) and osteoarthritis of the hip secondary to inflammatory disorders (19%). The most common presentation of the patients was with anterosuperior collapse of the femoral head. This led to flexion, adduction and external rotation deformity. 16 patients with unilateral hip affection had more than 2 cms limb length discrepancy and 51 hips had flexion, adduction and external rotation deformity greater than 20 degrees. All these hips were subjected to below mentioned intra-operative modifications.

The Harris Hip score at final follow up was 88±6.4 compared to pre-operative Harris Hip score of 35.4±5.2. As far as complications are concerned, there were three cases of superficial infection which were treated conservatively.

3.2 Pathoanatomy

We have encountered three types of hip arthritis secondary to avascular necrosis of femoral head. The radiological presentation includes anterosuperior collapse in 56/100 hips presented to us, Concentric/global collapse of head of femur in 25/100 hips and arthritic hip with inflammatory disorder conditions in 19/100 hips.We had observed that underlying pathology with duration of symptoms can affect the limb length discrepancy and range of movement. We have discussed problems encountered during surgical exposure, dislocation or relocation of hip and correction of deformity with different underlying pathology. We have also encountered flexion deformities in knee secondary to contracted hamstrings on medial side, contracted iliotibial tract on the lateral side due to chronic deformity of hip. The limb length discrepancy was found in such patients to be more than two centimetres. Overall, there are three presentation of hip arthritis affecting hip joint and surrounding soft tissues.

3.2.1 Various intraoperative findings associated in correlation underlying pathology

1. Anterosuperior collapse usually following AVN (56%)

Patients having anterior and superior collapse of head of femur usually are young. They have history of alcoholism or steroid intake or combination of both. The avascularity once it starts may progress in a year and half leading to FICAT Arlet Grade IV Presentations. These patients have usually flexion and adduction deformities. As they present earlier, limb length discrepancy is usually limited to 2 cm or less. There was flexion and adduction deformity found in forty hips with eight cases having external rotation deformity as well. The limb length discrepancy here is dependent on the chronicity of the symptoms. The longer the duration of symptoms, more is the joint destruction thereby limb shortening.

They may have bilateral affection as shown in Figs. 1a and 1b. So soft tissue contractures in such patients were found to be adductors, flexors and external rotators. Mean limb length discrepancy was found to be 2± 0.87 cm.

2. Concentric/global collapse of head of femur (25%)

This condition is usually seen where patients have symptoms which is persisting for more than two years and it is associated with avascularity of entire head of femur due to chronicity of symptom. We have observed such findings in sickle cell disease patients. Due to global avascular necrosis of femoral head collapse of the femoral head would be concentric and that would decrease the distance between acetabulum and trochanter without the protrusio acetabulum. This will reduce the distance between centre of femoral head to trochanter (neck length)

as well as reduced distance between ASIS (anterior superior iliac spine) to greater trochanter.

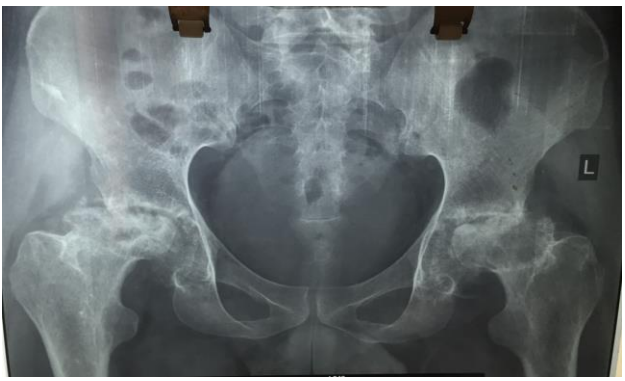

Fig. 1a. Pre operative radiographs anteroposterior views pelvis with both hips of 34 years gentleman with bilateral osteoarthrosis of hips showing anterosuperior collapse of femoral heads on both sides

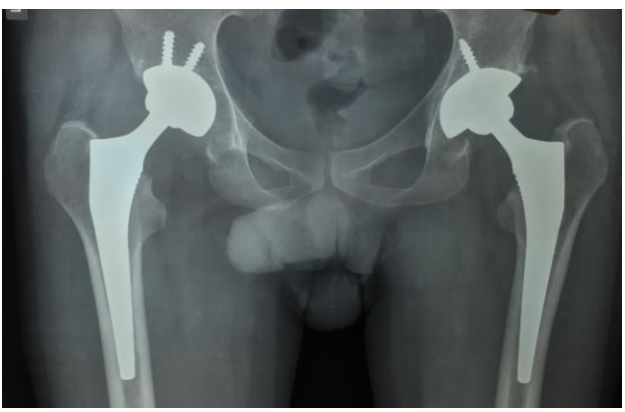

Fig. 1b. Post operative radiographs at 6 months with bilateral primary uncemented total hip arthroplasty

The chronicity of affection causes such contractures. In such patients there is global capsular contracture along with all muscles around the hip are contracted. So in such patients with unilateral hip affection, limb length discrepancy is seen to be more than two centimetres. A surgeon has to deal with global contracture of musculature around hip and its capsule as well. Even pre operative range of motion is remarkably reduced as seen in the Figs. 2a and 2b. Global musculature contracture would include contracture of gluteus maximus, gluteus medius, iliopsoas, tensor fascia lata, abductors, adductors, external rotators as well as

internal rotators of the hip. Stepwise release of soft tissue is also discussed in the subsequent section.

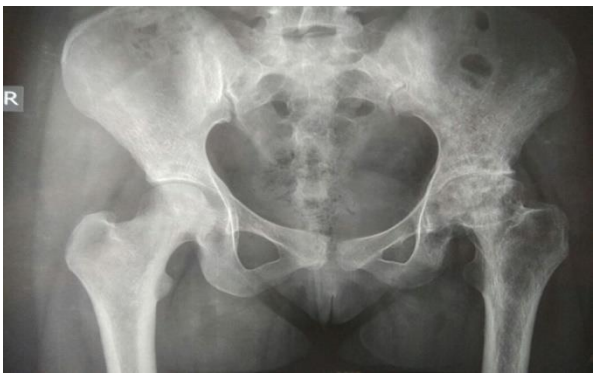

Fig. 2a. Pre operative radiograph anteroposterior view of pelvis with both hips of 23 years lady with left osteoarthrosis of hips showing concentric/global collapse of femoral heads on left sides

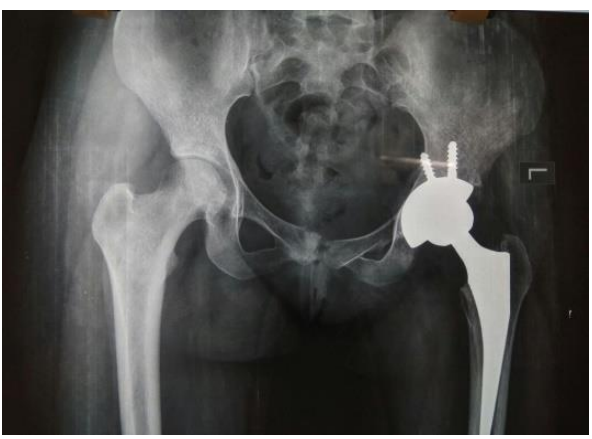

Fig. 2b. Post operative radiographs of the same patient showing equal vertical and horizontal offset on both sides with no limb length discrepancy

3. Inflammatory disorders: Rheumatoid Arthritis, Ankylosing Spondylitis & Tuberculosis of the Hip joint (19%)

Inflammatory disorders like rheumatoid arthritis, ankylosing spondylitis and tuberculosis may lead to arthritis of hip. Arthritis of hip due to inflammatory conditions is the third most common type of presentation. Among these arthritis conditions usually there is marked reduction in hip joint space with articular cartilage destruction. This presentation is different from secondary hip

osteoarthritis due to avascular necrosis of femoral head. Because this articular cartilage destruction leads to pain and muscle spasm which leads to stiffness. However these patients are unable to weight bear due to pain as this leads to rubbing of eroded articular cartilages. These groups of patients have restriction of movements due to muscle spasm. However if examined under anaesthesia, they may have reasonably good range of movements with lesser or no deformities.

3.3 Solutions

Above mentioned three different types of pathological presentations of arthritic hip and their anatomy may lead to intra-operative difficulty for performing primary total hip arthroplasty with standardized protocol. Some modifications are required depending upon the presentations of hip and its functional anatomy. These modifications are discussed below with each kind of presentation. It is essential that orthopaedic surgeons must understand the presenting symptoms and signs of each patient so that the pathoanatomy is defined preoperatively which simplifies intraoperative steps [31-33]. This simple pathoanatomical classification will definitely help young orthopaedic surgeons.

- **Modification during dissection –**

1. **For anterosuperior collapse**–As discussed earlier these group of patients will present with less then two centimetre limb length discrepancy along with flexion, adduction and external rotation deformity. Steps required for ideal release in such a patient will be adductor tenotomy in the groin; complete release of the external rotator muscles (piriformis, gemelli superior, gemelli inferior, obturator externus and quadratus femoris). Along with this we should also look for external rotation that is possible on table. Whenever we have corrected shortening of more than 2cm we have observed that full external rotation could not be achieved on table. This makes the reduction vulnerable for dislocation. In such a case the anterior attachment of gluteus medius over trochanteric ridge going down shaft of the femur to the anterior aspect on the iliac crest works as a culprit. Once we release anterior fibres of gluteus medius, we achieve full external rotation. If still it is restricted, anterior capsule must be released under vision. Only word of caution when we go for lengthening of more than three centimetres, sciatic nerve injury should be kept in mind [34-37].

2. **Concentric/global collapse of head of femur** – These patients usually present with more than two centimetre shortening and global soft tissue contracture. For correction of these limb length discrepancies, the surgeon should be very careful. We usually take posterior approach wherein external rotator release and posterior capsulotomy is preferred. However when we find that the hip has internal rotation contracture along with the global capsular contracture, we first do capsulotomy anteriorly at the distal attachment of the capsule over intertrochantric line along with the release of anterior vertical insertion of gluteus medius

over the descending shaft. After doing this we go for release of posterior structure. These facilitates better exposure in the posterior approach. Special attention has to be given to abductor muscle contracture and superior capsular contracture. We usually follow a policy of doing gluteus maximus tendinous insertion tenotomy in such patients having more than two centimetres shortening and this also facilitates a very comfortable relaxed posterior approach in such group of patients. Whenever there is shortening due to abductor muscle contracture, it becomes difficult to achieve horizontal as well as vertical offset alignment during the operation. With persistent reduction in distance between acetabulum and trochanter even after global capsular release, it becomes difficult to perform reduction after implantation due to reduction in neck length with abductor contracture. This may lead to either fracture of trochanter on table while doing the reduction of the prosthesis or overstretching if the release is not planned well. As far as abductor shortening is concerned we go for VY lengthening of abductor muscle or osteotomy of greater trochanter can be done which would have to be reattached with tension band wires. This is usually decided only if reduction is very difficult and will be required in few patients. Iliospoas release from lesser trochanter may have to be performed, if post-reduction there is persistent flexion deformity more than 10º.

If there are flexion, abduction deformity with external rotation deformity with equal limb lengths post-reduction, it is suggestive of either contracture of tensor fascia lata or the abductors. Tight tensor fascia lata may also present with flexion deformity at the knee joint. In these patients, release of tensor fascia lata at the anterior superior iliac spine as well as multiple transverse releases of tensor fascia lata at the trochanteric level will work. It will be prudent to mention here that in distal half of incision we separate out TFL from surrounding structure and cut it transversely.Thereby we avoid cutting the muscular part of tensor fascia femorale. The transverse tenotomy relaxes distal TFL fascia which automatically corrects flexion as well as abductor contracture. Please remember,this contracture is evident post-reduction as the limb length is corrected. If the deformity persists even after the release of tensor fascia lata, abductor release becomes necessary.

- **Deformities and release of structure**

The stepwise technique for global release include: 1. Adductor Tenotomy 2. External rotator release 3. Gluteus Medius Anterior Fibres Release. 4. Anterior Capsule Release 5. Gluteus maximus tendinous insertion tenotomy 6. Posterior Capsule Release. 7. TFL (Tensor Fascia Lata) release 8. Iliopsoas Release 9.Abductor Release (very rare). Please note that after each step, it is important to confirm whether further steps are required.

- **Repair of the soft tissue following global release**

External rotators should be sutured with greater trochanter, anterior and posterior capsule should be closed to avoid post operative dislocation, abductors are

repaired with VY plasty, gluteus maximus insertion sutured with femoral shaft. We prefer not to repair adductor tenotomy, iliopsoas and gluteus medius releases.

3. **Inflammatory disorders** – These patients present with limited range of motion of hip in all planes due to muscle spasm and pain. And if such patients are examined under anesthesia irrespective of pre-operative deformity, the movement may be full on table before surgery. With available range of movement under anesthesia for such a patient surgical release is planned as per the need. This patient's bone is however osteoporotic and acetabulum may have protrusio in ankylosing spondylitis. In such a patient preoperative planning for correct placement of cup is essential. If there is superior defect / medial defect like pseudo acetabulum, protusio then the bone graft from the femoral head is must.

- **Post implantation assessment and modification –**

Assessment of movements after implant fixation in an arthroplasty is must which could help us in following situations:

1. When external rotation is restricted release fibres of gluteus medius which is a strong medial rotator having attachment on anterolateral surface of greater trochanter needs to be done to facilitate external rotation post surgery. Since external rotation of the hip is essential for stability of joint.
2. Persistance of abduction and flexion deformity of more than five degrees:Post implantation release of anterior fibres of gluteus medius followed byclosed tenotomy of tensor fascia lata is advised.
3. If flexion deformity of the hip persists after implantation and reduction, then release of iliopsoas muscle from lesser trochanter insertion site has to be done.
4. The hip should be stable, having range of movements with atleast 90º flexion, 45º adduction-abduction, 30º internal and 60 ºexternal rotation. The limb length should be equal on both sides.

In all patients with shortening >2 cm tenotomy of gluteus maximus tendinous part is done along with superior and anterior capsular releases. The anterior capsule is released by going anteriorly with posterior approach by externally rotating the limb. If internal rotation deformity along with flexion deformity is present anterior capsulotomy may be necessary. After implantation two things need to be observed: a) Restriction of external rotation – if persists then fibers of gluteus medius attached on anterolateral surface of greater trochanter has to be released. b) Abduction and flexion deformity >5 degree post implantation release of tensor fascia lata by closed tenotomy followed by above procedures need to be carried out. The limitation of our study is short term follow up and a small series.

4. CONCLUSION

We hereby present a unique pathoanatomical group classification along with various clinical presentations. Broadly, it includes: a. Avascular Necrosis of femoral head with only anterosuperior collapse, b. Avascular Necrosis of femoral head with global collapse and contracture and c. Inflammatory/infective disorders. Surgical problems encountered during the operation and their solutions are described for each type of pathoanatomy. This will help orthopedic surgeons to anticipate and prepare well in advance while performing total hip arthroplasty.

Operating surgeon should have precise knowledge of hip anatomy, associated soft tissue contracture and deformities before releasing deforming contractures to avoid intraoperative problems and reduce operative time. Further future studies and large sample size are required to validate our results.

ETHICAL APPROVAL

As per international standards or university standards written ethical approval has been collected and preserved by the author(s). (SVIEC /ON/MEDI/BNPG/15/ D16207).

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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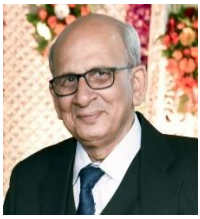
Biography of author(s)



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Chapter 8
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Excision of a Gingival Fibroma in the Anterior Maxillary Region of an Edentulous Patient with Diode Laser

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ABSTRACT

Gingival fibromas are benign growths of the gingiva with unique histological features. Clinically, they frequently appear as solitary, smooth, hard nodules that may or may not have surface ulcerations. In this report, we discuss the case of a 60-year-old female patient who presented with a nodular gingival mass located on the edentulous alveolar ridge in the maxillary left canine region. The diagnosis of gingival fibroma, which is a gingival pathology with unique histological features, was made based on clinical and histopathological examinations. The lesion was successfully removed with a diode laser in a chairside procedure.

Keywords: Diode laser; gingival fibroma; gingival growth.

1. INTRODUCTION

Gingival fibroma (GF) is a benign nodular growth of the gingiva classified histopathologically as a distinct clinical entity [1]. Previously, these lesions were commonly termed irritation fibromas [2], presumed to arise from chronic local trauma of the oral mucosa; in some cases, they were medication-related overgrowths [1,3,4]. GF involves the maxillary and mandibular mucosa, and it affects both sexes [2].

Clinically, GF often presents as a solitary, smooth, firm nodule ranging up to 2 cm. Surface ulceration may occur [1].

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A definitive diagnosis of GF is made histologically, with a characteristic spiral arrangement of cellular components within a fibromyxoid stroma lacking inflammation, vascularity, or odontogenic epithelium [1].

GFs should be differentiated from typical reactive fibrous growths like fibroepithelial polyps, traumatic fibromas, focal fibrous hyperplasia, peripheral ossifying fibroma, peripheral odontogenic fibroma, etc. These growths are characterized by dense collagenous tissue and a low cellular density [1-4]. Standard therapy is surgical excision with extension of margins. The recurrence rate is low, reported under 10% [1,3-5].

In this report, we describe a case of GF in the anterior maxillary region, including the diagnostic approach and surgical management. The aim is to contribute an additional well-documented case to the growing literature on this recently characterized gingival pathology.

2. CASE PRESENTATION

The patient is a 60-year-old female with an unremarkable medical history. Routine hematological tests, including hemoglobin, bleeding time, clotting time, and random blood glucose, were within normal limits. Her dental history is significant for recent extraction of the maxillary left canine and lateral incisor by her general dentist, where a small gingival growth was present before extraction that progressively enlarged after.

The patient presented to our clinic with a 2 cm smooth, nodular gingival mass located on the edentulous alveolar ridge in the maxillary left canine region. It should be noted that the patient does not wear a denture. The lesion was predominantly pink, with some focal red regions. There was no evidence of ulceration (Fig. 1).

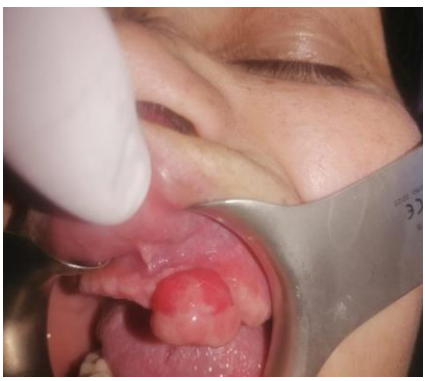

Fig. 1. Intraoral photograph showing a nodular gingival mass located on the edentulous alveolar ridge in the maxillary left canine region

After obtaining written consent from the patient, the lesion was surgically excised using a diode laser (Medency Technology Company- Vicenza, Italy) under local anesthesia and standard precautions (Fig. 2).

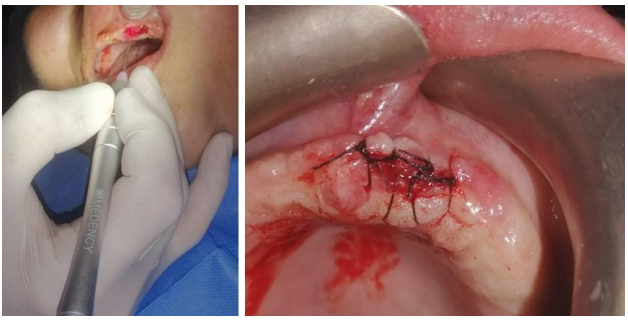

Fig. 2. Intraoral photographs during and after resection of the lesion by diode laser

Then the specimen was then sent for histopathological examination, which showed spindle-shaped cells with regular nuclei arranged in long bundles within an abundant collagenous matrix. The overlying epithelium was focally parakeratotic and extensively eroded, exposing granulation tissue. The final diagnosis was peripheral eroded gingival fibroma (Fig. 3).

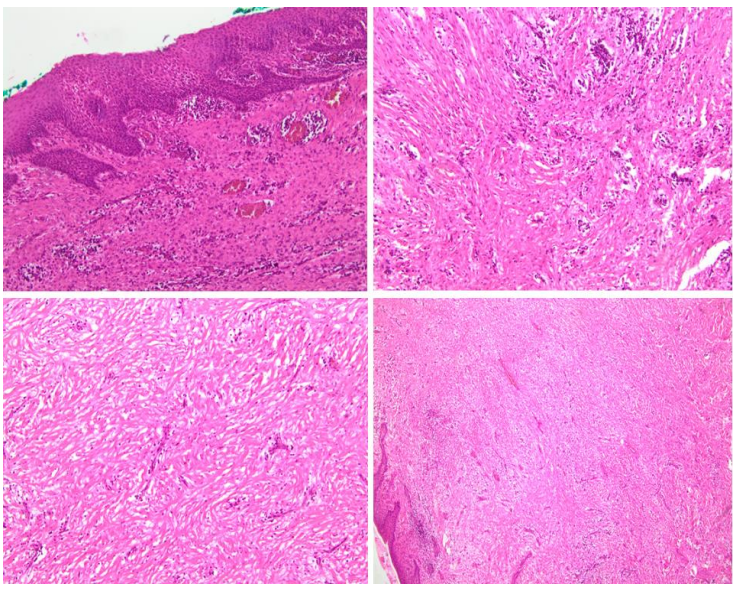

Fig. 3. Histological features of the lesion

3. DISCUSSION

This case provides noteworthy documentation of GF, a recently described and poorly characterized lesion before the Bawazir et al. study in 2021 [1], as well as illuminating some distinguishing traits while also demonstrating the efficacy of laser excision.

In the scientific literature, a high predilection in female patients with GF was noted [1,4]. Additionally, the majority of these lesions were found on the anterior maxillary buccal mucosa [2], distributed on the anterior incisor region (66.7%), and the canine- premolar region (11.7%) [1]; our case involves a female with a lesion on the buccal mucosa around the canine area.

GFs pose challenges in clinical differentiation from other reactive gingival lesions, such as pyogenic granuloma, peripheral ossifying fibroma, peripheral odontogenic fibroma, and peripheral giant cell granuloma, which clinically appear similar but possess distinctive histopathological features.

In fact, among the hallmark histological features of GF that help reliably discriminate it from the other reactive lesions cited above is a prominent fibromyxoid background along with a lack of odontogenic epithelium and/or any calcification [1,6-8].

As for other reactive gingival lesions, conservative surgical excision with safety margins and down to the periosteum is the preferred treatment; however, this method presents challenges in intraoperative bleeding control, suturing, and the potential for postoperative edema [9]. In our case, we successfully employed a diode laser as a safe and effective alternative, demonstrating reduced bleeding, inflammation, and scarring compared to conventional scalpels [10]. Alternatively, the CO2 laser could have been utilized, offering advantages such as minimized bleeding, decreased postoperative discomfort, and minimal swelling [11]. Nd:YAG could also have been considered for its superior coagulation characteristics and reduced risk of bleeding in oral lesion removal [12].

According to many authors, the recurrence rate is minimal, ranging from 6.7% to 8% [1,4]. Additionally, continued monitoring is imperative given the limited literature on GF behavior.

4. CONCLUSION

This report presents a GF confirmed by histopathology. Diode laser excision enabled effective treatment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 9
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Research on Liver Organoid: Present Situation, Limiting Factors and Future Therapeutical Potential in Pediatric Diseases

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ABSTRACT

Scientists often use organoids to research diseases. Organoids are three-dimensional, organ-like cell assemblies in which different cell types have organized themselves in a way that is approximately typical for the corresponding organ in the body. They show three characteristics: self-organization, multicellularity and functionality. This study concentrates on liver organoid research and its future role in different pediatric diseases. The range of organs that can be studied with organoids is growing rapidly and includes the brain, intestine, kidney, stomach, pancreas, lung, liver, prostate, esophagus, gallbladder, and the female reproductive tract, among others, and also the embryo. Organoids enable the scientific study of human development, physiology and pathology on a scale. Organoids are grown either from pluripotent stem cells or from tissue-specific adult stem cells. Adult stem cells are present in a large number of tissues and are responsible for renewing the cells in these tissues. They can only give rise to the cell types that are present in the particular tissue, the stem cell of the intestinal epithelium only produces cells of the intestinal epithelium, but not muscle cells or nerve cells. They are thus multipotent. Today, it is possible to reconstruct organ-like tissue organoids in the laboratory. Stem cells are thereby induced to differentiate by molecular signals and grown in culture systems that promote their three-dimensional self-organization. Rapidly developing organoid technology makes it possible to phenotypically copy cell structure. To some extent, this is also true for the functions of various human

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organs (for example, brain, thyroid, thymus, intestine, liver, pancreas, stomach, lung, kidney) and even early-stage embryos. As near-physiological 3D culture systems, organoids open up new possibilities to study the development of healthy and diseased organs and offer great potential for translational research. Further research in the field of paediatrics will show further development of *in vivo* use of organoids in the future, especially in liver diseases in childhood.

Keywords: Organoid; pediatric; liver; hepatocyte; child; future research.

1. INTRODUCTION

Organoids are simple tissue-engineered cell-based in vitro models that recapitulate many aspects of the complex structure and function of the corresponding *in vivo* tissue [1,2]. Scientists often use organoids to research diseases [3-58]. The modern term organoid refers to cells growing in a defined three-dimensional (3D) environment in vitro to form mini-clusters of cells that self-organize and differentiate into functional cell types, recapitulating the structure and function of an organ *in vivo* (hence, also called “mini-organs”) [59]. These clusters of specific cell types are created in the laboratory from human stem cells. Depending on the nutrient solution and treatment, they form three-dimensional organ-like structures from several cell types [1-56]. They can be used to more realistically reproduce malfunctions or developmental steps because they are more similar to the human body than one-dimensional cell cultures of individual cell types [2,4,23,34,56]. In particular, cells in brain organoids can even mature to the point where they resemble those of a postnatal brain [60]. The brain cell clusters mirror the genetic and structural changes in the brain of a newborn. Previously, researchers had assumed that the organoids were only suitable for studying prenatal brain development. Organoids could also be suitable at the advanced stage to study neurological diseases that develop only with the more complex development of the brain. Concerning the liver organ, it has been known since Aristotle that the human liver has the greatest regenerative capacity of all organs in the body and can regrow even after an amputation of 70%. This makes transplantation by liver donors possible. The molecular mechanisms by which adult liver cells trigger regeneration are still largely unknown. About 29 million people in Europe suffer from chronic liver diseases such as cirrhosis or liver cancer [60]. They are a major cause of disease and mortality, with liver disease contributing to about two million deaths worldwide each year. Currently, there is no cure and liver transplants are the only treatment for liver failure. Scientists in the field of molecular cell biology and genetics are investigating the biological basis of liver regeneration in humans. In 2013, Huch and Clevers developed the first liver organoids-miniature liver tissues that were created from mouse liver cells in a Petri dish in the laboratory. The researchers even succeeded in transplanting the organoid into a mouse, where it could take over liver functions. In 2015, they successfully transferred this liver organoid technology to culturing a human liver in a Petri dish based on human liver samples. The two most important functional cells in the adult liver are the hepatocytes, which perform many functions in the liver, and the ductal cells, which form the network of tiny ducts through which bile

is directed to the intestine [60]. These work together with other supporting cells, such as the blood vessels or the mesenchymal cells. To build liver organoids, researchers initially used only ductal cells of the bile duct. In a healthy liver, there are a certain number of contacts between the ductal cells and the mesenchymal cells that signal the ductal cells not to proliferate and just stay as they are. Once the tissue is damaged, the mesenchymal cells reduce the number of contacts they have with the ductal cells so that the ductal cells can proliferate to repair the damage. From their observations, the researchers concluded that it is the number of cell contacts, rather than the number of both cell types, that determines how many cells are produced to repair the damaged tissue. Too many touches by mesenchymal cells mean that fewer or no new ductal cells are produced, while fewer touches mean that more cells are produced [60]. Organoids are three-dimensional structures of cells generated from stem or progenitor cells *in vitro*. They resemble organs *in vivo* in terms of the cell types they contain, their spatial arrangement and specific functionality [4,7,12,26,52]. Their development is often characterized by the term "self-organization". This is understood as a process of formation of complex structures from initial cells by interactions of the cells with each other and between the cells and their environment. Stem cells are cells that can give rise to further stem cells as well as specialized cells (ability to differentiate) by division. Progenitor cells, on the other hand, are descendants of stem cells already committed to the formation of specific cell types. The range of organs that can be replicated in this way in different species is now very wide and includes organs and cell types that have arisen from the cells of all three cotyledons. Cotyledons are the three cell layers hat form during embryonic development and that can give rise to different tissues in the course of further development [60]. The three cotyledons contain, so to speak, the developmentally most distant groups of cells. Since organoid technology can already reproduce these greatest possible cellular differences, it is assumed that in principle organoids of all organs can be produced. However, one organoid does not always represent the entire organ: frequently, several organoids reproduce different aspects of individual organs, thus making them accessible to experimental scientific research. Their potential for application in various fields, especially biomedical research and therapy, is promising his ranging from basic research, *in vitro* investigation of organ development and disease research, to use as test systems for drug development and toxicity testing, to cell, tissue and organ replacement within regenerative medicine [60]. Research on organoids raises great hopes and opens up new perspectives, especially for the endeavour of increasingly personalized medicine and in the field of paediatrics [1-56].

2. PRESENT SITUATION OF ORGANOID RESEARCH

The range of available organoids is growing rapidly and includes replicas of the brain, intestine, kidney, stomach, pancreas, lung, liver, prostate, esophagus, gallbladder and the female reproductive tract as well as the embryo (so-called embryoids) currently, organoids are primarily used by researchers as model systems for different organs to better study their development, functioning and diseases [1-56]. In addition to their utility in basic research, they are used for

drug development and toxicity testing [60]. In the Netherlands, organoids are also already part of the healthcare system as patient-derived organoids from cystic fibrosis patients for pre-testing of drugs. However, they also raise questions that have so far been little discussed in Germany. These include, for example, questions about the transferability of research results on organoids to corresponding organs *in vivo* or whether it might be possible in the future to counter the shortage of donor organs by replacing organs in the form of organoids. However, major technical hurdles and unresolved scientific questions still stand in the way of this vision. Another ethically controversial issue, for example, is how the possible development of consciousness in the increasingly complex brain organoids should be judged, involving questions about the measurability of mental and cognitive processes on the one hand and possible claims for protection on the other [60].

3. FUTURE THERAPEUTICAL POTENTIAL IN PEDIATRIC DISEASES

Organoids enable the scientific study of human development, physiology and pathology on a scale and with a level of precision previously unheard of. To date, scientists have explored this using animal models and two-dimensional human cell culture models. Appropriate approaches have led to countless important discoveries, but have specific limitations: *In vivo* animal models are not ethically sound, are costly and time-consuming; moreover, they only imperfectly replicate human physiology, and their complexity can make it difficult to determine cause and effect in experiments. Conventional human 2-D cell culture models, on the other hand, are often too simple because they often contain cells of only one cell type [60]. Moreover, 2-D cell culture models are typically derived from patient cancer tissues or induced into a cancer-like state by viral oncogenes, which allows for unlimited propagation of these models *in vitro*, but can also lead to genomic instability and differences in these models compared to their *in vivo* counterparts.

Organoids, on the other hand, can also be generated from healthy human cells, contain many of the cell types found in an organ, and exhibit a stable genotype-phenotype relationship as well as aspects of human organ architecture, physiology, and function.

4. DISCUSSION

Organoids enable the scientific study of human development, physiology, and pathology at a scale and level of precision not seen before. To date, scientists have explored this using animal models and two-dimensional human cell culture models [1-56]. Appropriate approaches have led to countless important discoveries, but have specific limitations: *In vivo* animal models are not ethically sound, are costly and time-consuming; moreover, they only imperfectly replicate human physiology, and their complexity can make it difficult to determine cause and effect in experiments [60]. Conventional human 2-D cell culture models, on the other hand, are often too simple because they often

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5. CONCLUSION

Currently, organoids, particularly lung, kidney, liver, pancreas, and intestinal organoids, are being used for COVID-19 research, especially for modelling certain disease processes and for screening existing drugs for other diseases for efficacy against Sars-Cov-2 [60]. They are also important for cancer research [1-56]. Further research in the field of paediatrics will show further development of *in vivo* use of organoids in the future, especially in liver diseases in childhood [1-56].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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He was born in 1968. He is a German pediatrician and studied human medicine from 1989 to 1996 at the Universities of Münster (WWU), at the Rudolphina in Vienna during a year of study abroad, and at the Ludwig-Maximilian University (LMU) in Munich. He obtained student internships during medical studies several times in Switzerland as well as in the USA. He completed his medical studies in 1996 with the 3rd state examination in Munich. He received his doctorate with the topic of biliary atresia in childhood at the Pediatric Surgical Clinic of the Hauner Children's Hospital of the LMU Munich under Professor Dr. Kellnar. He received his full medical license at the University of Munich in 1998 and his doctorate in medicine in 1999. He worked as an intern at the Pediatric Surgery Clinic of the Hauner Children's University Hospital in Munich. He received his first research assistant position in 1999 in the Pediatric Surgery Department of the Clinic for Surgery under the then-head of the clinic, Professor Margreiter, and Professor Menardi for the area of pediatric surgery. Later, he obtained pediatric surgical training in Oberhausen under Professor Pieper. Following this further training phase, he moved to Switzerland to further his own development at the University Children's Hospital of Zurich, initially under Professor Urs Stauffer, and later under Professor Martin Meuli, who had succeeded Professor Stauffer. He was scientifically involved in the laboratory for tissue engineering of Prof. Reichmann, first animal experimental studies as well as the center for severe burn injuries of the Children's Hospital Zurich under Professor Schiestl. Subsequently, he received further training in pediatrics at the Children's Hospital Ahlen under Professor Krüger and completed further clinical and anthroposophical training in pediatrics under Professor Längler at the University of Witten-Herdecke. He completed his residency in pediatrics under Professor E. Harms. He took over the pediatric department in Gronau (Westf.) in April 2009. He completed a part-time master's degree program in complementary medicine at the European University Viadrina in Frankfurt (Oder) and graduated in 2013 with the master's thesis on HPV vaccinations and the master's examination in Bad Honnef and received the academic degree of Master of Arts (M.A.). In the further course, he set up one of the largest pediatric day departments in Westmünsterland and continued clinical research at the Ped Mind Institute. In addition, he received regular continuing education diplomas from the Association of Statutory Health Insurance Physicians and completed a university degree in sleep medicine and sleep culture at the Apollon University of Applied Sciences in Bremen in 2019. In his professional career, he has published approximately 180 scientific publications and books in the field of pediatrics and pediatric surgery (as of 1/23). His book “Checkliste Pädiatrie und Neonatologie”, 2nd edition, is in the press and will be published in September 2024. He was appointed as a Visiting Professor in Pediatrics in September 2022 by the Dean of the School of Medicine, Shangluo Vocational and Technical College, University of Shangluo, China, where he holds a teaching position.

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Chapter 10
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Pivotal Roles of APN/AdipoRs Signaling in Maintaining Ocular Homeostasis and Protecting against Eye Ailments

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ABSTRACT

This comprehensive review critically examines the latest findings and breakthroughs that underscore the pivotal roles of APN/AdipoRs signaling in maintaining ocular homeostasis and protecting against eye ailments. Explaining the complex interactions between ocular diseases and the flexible fat-associated adipokine adiponectin (APN) has garnered a lot of attention in recent years. Discovering the complex connection between aging eye problems and adipocytokine and its receptors (AdipoRs) has become an exciting area of medical research. Here, we meticulously explore the intriguing mechanisms by which APN protein influences retinal function and overall visual acuity. Drawing from an extensive array of cutting-edge studies, the article highlights APN's multifaceted functions, ranging from anti-inflammatory properties and oxidative stress reduction to angiogenic regulation within retinal and macula tissues Targeting common aging eye diseases with treatment approaches could be made possible by the role that APN/AdipoRs play in mediating these effects. Furthermore, the interaction between APN signaling pathways and age-related macular degeneration (AMD) is unraveled in this review. The single-cell RNA-seq results validate the expression of both the receptor isoforms (AdipoR1/R2) in retinal cells. The transcriptomic analysis showed lower expression of AdipoR1/2 in dry AMD pathogenesis compared to healthy subjects. The inhibitory adiponectin peptide (APN1) demonstrated over 75% suppression of choroidal neovascularization (CNV), whereas the control peptide did not exert any inhibitory effect on CNV. The

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elucidation of these relationships fosters a deeper understanding of adipose tissue's profound influence on ocular health, presenting new prospects for personalized treatments and preventative measures. Because APN1 inhibits CNV and leakage, it can be used to treat human AMD. In conclusion, this review provides a captivating journey into the enthralling world of APN, intertwining the realms of adipose biology and ophthalmology in aging. The remarkable connection between fat, in the form of adiponectin, and t654r4dexzsight opens exciting avenues for advancing eye health and improving patient outcomes.

Keywords: Obesity; adiponectin; adiponectin receptors; age-related macular degeneration; dry AMD; wet AMD; choroidal neovascularization; angiogenesis; inflammation.

1. INTRODUCTION

Adiponectin, an adipokine secreted by adipocytes, is a well-known homeostatic factor for regulating glucose levels, lipid metabolism, and insulin sensitivity through its anti-inflammatory, anti-fibrotic, and antioxidant effects [1a]. APN plays a crucial central role in various vital bodily functions such as managing glucose and fatty acid metabolism [1,2]. It also contributes to maintaining glucose and lipid balance [3], overall energy regulation [4], immune responses [5], and the effects of aging and metabolism [6–8]. The reduction of APN expression levels observed in obese patients, has been related to an increase of tumor onset risk. Several studies demonstrated that APN, beyond its actions in metabolic responses such as energy metabolism regulation and insulin-sensitivity, has pleiotropic effects in cancer. Although literature data on the role of APN in carcinogenesis is conflicting, the most accredited hypothesis is that APN has a protective role, such as anti-inflammatory, anti-proliferative and pro-apoptotic effects, avoiding the development and progression of several malignancies, such as breast, colon, prostate, liver and endometrial cancers [8a]. Notably, APN extends its safeguarding influence on ocular tissue [8,9]. The retina, a metabolically very active component often outpacing even the brain's metabolic rate, triggers blood vessel growth and regression due to its high-energy needs. APN has also demonstrated protective effects against multiple retinal disorders, including diabetic retinopathy (DR) [9, 9a], choroidal neovascularization (CNV) arising from age-related macular degeneration (AMD) [10], and other additional retinal complications [11].

Neovascular AMD is a complex retinal condition where an individual's genetic disposition is influenced by the effects of age and environmental stressors. These factors cascade in a series of signaling pathways that involve inflammation, oxidation, and/or angiogenesis within the retinal pigment epithelial (RPE) cells and choroidal endothelial cells (CECs) [8]. Ultimately, this process results in vision loss due to the advancement of CNV. Navigating the world of AMD, we encounter its dual personas: the wet and dry types. Unveiling its dramatic impact, the wet form takes the spotlight, emerging as a chief instigator of irreversible blindness and the complete eclipse of central vision among the elderly [9a]. The hallmark of this drama is CNV [8,10]. In contrast, the dry type, though not synonymous with total

blindness, casts a shadow over central vision, posing challenges in reading, driving, and perceiving the world around. As the curtain rises on advanced stages, dry AMD can take a perilous turn, progressing into geographic atrophy (GA) or even evolving into its wet counterpart, both orchestrating a symphony of severe vision loss [10]. Initiating a cascade of events, retinal hypoxia triggers an upsurge in metabolic demands, setting off signaling pathways that endeavor to tap into new vascular resources, ultimately culminating in the eye's neovascularization [10]. The literature hints at a shift in the balance of two predominant circulating adipokines, APN and leptin, pivotal players in metabolic modulation across diverse tissues [9a]. This dynamic duo might play a role in driving the progression of neovascular eye conditions [9]. Further insights point to the heightened release of leptin, a hormone originating from adipocytes, as a harbinger of disrupted energy equilibrium, increased oxidative stress on vascular endothelial cells (ECs), and consequent dysfunction of these cells, ultimately contributing to retinopathy [12]. In parallel, another metabolic influencer, primarily sourced from adipocytes, APN, joins the orchestra of metabolic irregularities in the retina. The levels of circulating APN are intricately tied to DR [13,14], the development and advancement of premature retinopathy [15], and age-related macular degeneration [16]. This correlation is underscored by research, including studies involving laser-induced choroidal neovascularization [17,18] and a rodent model of oxygen-induced proliferative retinopathy, where higher circulating APN levels correlate with suppression of pathological vascular proliferation [19]. Embarking on a journey through ongoing investigations and pertinent studies, we delve into this burgeoning realm of APN/AdipoRs, dissecting their roles in the intricate landscape of retinal neovascular disorders [9a].

2. UNVEILING ADIPONECTIN/AdipoRs PHYSIOLOGICAL ROLES

2.1 Unlocking the Mysteries of APN/AdipoRs: A Journey through Discovery, Structure, and Forms in Circulation

During the mid-1990s, APN's discovery unfolded as a harmonized effort across four research laboratories [20–23]. In contrast, the identification of APN receptors denoted as AdipoRs, underwent a protracted gestation period, finally coming to fruition in 2003 through pioneering work by Yamauchi et al. [24]. This milestone was achieved by selectively extracting two strongly associated seven-transmembrane receptors isoform, AdipoR1 and AdipoR2, from human skeletal muscle [25]. The foundational architecture of APN is constructed with a carboxy (C)-terminal globular domain paired with an amino (N)-terminal collagen-like domain [26, 9a]. The AdipoRs adopt the form of integral membrane proteins, where the N-terminus faces internally, and the C-terminus faces externally—a distinctive arrangement that diverges from the topology and role of other recognized G protein-coupled receptors (GPCRs) [27]. In a pattern of ubiquity, APN and AdipoRs manifest their presence across diverse tissues [28]. In addition to the dynamic duo of AdipoRs, APN extends its influence through interaction with the receptor T-cadherin, although, at present, its role seems less pivotal in comparison to AdipoRs [29].

The obesity-related peptide APN assumes a complex structural configuration and circulates within the bloodstream in different molecular forms: a trimer, hexamer, and a higher molecular weight (HMW) oligomer. These diverse APN variants exhibit varying levels of biological activity, with HMW oligomer APN being identified as the biologically energetic iteration of this hormone [30]. Interestingly, in specific scenarios, the HMW form has demonstrated superior insulin-sensitizing properties when compared to trimers or hexamer forms. In addition to its intricate structural diversity, APN undergoes glycosylation, a crucial post-translational modification necessary for maintaining its functionality [9a]. Its concentration in circulation typically ranges from 3 to 30 μg/mL in both humans and rodents, making it one of the most abundant adipokines present in the plasma [31,32].

2.2 Tissue Distribution, Mechanism, Physiological and Pathological Relevance of APN/AdipoRs Pathway

Primarily originating from adipocytes, APN is expressed in various locations in addition to plasma and imparts favorable influences on several metabolically demanding organs and cell types [33,34]. These include liver parenchymal cells (PCs), such as hepatocytes [35], skeletal muscle and myocytes [36], the brain [37], blood vessels [38], and reproductive organs in both males and females [6,39], as well as ocular tissues [9,40]. AdipoR1 and AdipoR2 exhibit broad and abundant expression, not limited to skeletal muscle and liver tissues but also extending to macrophages [41], the hypothalamus [42], white adipose tissue [43], reproductive tissues [44,45], and the retina [46]. In both in vitro and *in vivo* studies, AdipoRs have emerged as pivotal mediators of APN signaling [8]. APN engages with its two well-established, distinct cell-surface receptor variants, AdipoR1 and AdipoR2 [24]. Furthermore, AdipoR1 plays a more prominent role in initiating the AMP-activated protein kinase (AMPK) pathway, leading to the inhibition of hepatic glucose production and an increase in fatty acid oxidation. On the other hand, AdipoR2 is primarily associated with activating the peroxisome proliferator-activated receptor alpha (PPARα) nuclear receptor pathways, which in turn promote fatty acid oxidation and mitigate tissue inflammation and oxidative stress [47]. In metabolic organs targeted by insulin, such as the liver and skeletal muscle, the expression of AdipoRs significantly increases during fasting conditions compared to refed conditions in rodent models [9a]. Additionally, *in vitro* studies have revealed that insulin reduces the expression of AdipoRs through the phosphoinositide 3-kinase/FoxO1-dependent pathway [48]. The levels of APN in circulation and the presence of AdipoR1/R2 expression in metabolically active organs remain lower in obese and diabetic individuals as compared to healthy and lean individuals [49–51]. There is a significant reduction in APN concentrations among obese patients with Type 2 diabetes (T2D) and infertility [52]. In most cases, insulin acts as a stimulating factor, while tumor necrosis factor-alpha (TNF-α) serves as an inhibitor of APN signaling and secretion [53]. Additionally, it exerts a controlled influence on inflammatory responses by mitigating the production and functional activity of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) within macrophages through the inhibition of NF-κB activation, as elaborated earlier [54]. Furthermore, APN is recognized for its specific actions in regulating metabolism and developing insulin sensitivity [9a].

APN also plays a vital role in managing multiple physiological processes, including glucose utilization, lipid biosynthesis, energy homeostasis, and inflammatory and retinal function [15,55, 9a]. Lack of APN secretion and expression globally or locally leads to insulin resistance, glucose intolerance, and hyperlipidemia in rodents [56,57]. APN along with other adipocytokines plays a primary pathophysiological function in the interaction between metabolism and reproduction and may be associated with the detrimental effect of aging on male reproductive actions [7,39]. The use of APN supplementation could potentially serve as a crucial therapeutic approach for addressing reproductive disorders associated with obesity, such as male and female infertility [6,58]. Knockdown experiments involving the ADIPOQ gene in skeletal muscles demonstrate the pivotal function of AdipoR1 in orchestrating various processes, including but not limited to β-oxidation, AMPK and PPAR pathway activation, glucose uptake [59]. AdipoR1 plays a role in elevating the phosphorylation of AMPK within the liver, consequently affecting the gluconeogenesis process. Concurrently, AdipoR2 is accountable for the removal of reactive oxygen species (ROS) and the initiation of nuclear receptor PPAR activation, along with the downstream modulation of target genes associated with β-oxidation [9a]. The emergence of nonalcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH) accompanied by fibrosis and inflammation in obese rats fed a high-fat/high-cholesterol diet highlights the involvement of AdipoRs in regulating hepatic fatty acid metabolism. The decreased expression of AdipoRs isoforms during NASH was linked to lower levels of PPARα and AMPKα 1/2. Furthermore, specific tissues played a crucial role in determining these effects. AdipoR1 in the liver played a role in activating AMPK, while AdipoR2 was actively engaged in activating PPARα, resulting in heightened insulin sensitivity [60,61]. Due to their proficiency in establishing cross-organ communication and its lipid sequestration capabilities, APNs assumes an essential role in the preservation of lipid and glucose homeostasis [9a]. In a particular investigation, the overexpression of adiponectin and its associated receptors (AdipoRs) yielded numerous favorable outcomes, notably the reduction of visceral adiposity, amelioration of inflammatory responses, and mitigation of hepatic fibrosis [62].

3. UNLOCKING THE POTENTIAL OF APN/AdipoRs AS METABOLIC REGULATORS IN RETINAL DISEASES

Recent investigations have explored the existence of APN/AdipoRs in ocular tissues [46,63], with a particular focus on its role in conditions such as DR, retinopathy of prematurity, the preservation of hypoxia-induced retinal neovascularization, photoreceptor integrity, retinitis pigmentosa, and AMD within the context of ocular pathophysiology [9a]. As previously discussed, it is important to note that the retina is among the most metabolically demanding tissues in the human body, and photoreceptors, in particular, house a greater number of mitochondria even relative to cardiomyocytes [64]. The retina is supplied with essential nutrients and oxygen through its vascular network. Premature loss of these blood vessels can induce hypoxia and insufficiency of energy substrates, both of which are recognized as pivotal factors in instigating angiogenesis within retinal tissue [9a]. Hypoxia leads to a reduction in the activity of prolyl hydroxylase,

an enzyme well known for its capacity to rapidly degrade the hypoxia-inducible factor (HIF)-1 protein under normal oxygen conditions. Elevated levels of HIF-1 protein, in turn, initiate the expression of angiogenic factors, most notably vascular endothelial growth factor A (VEGFA) [65]. It is worth noting that specific metabolic pathways, independent of HIF-1, can also influence the regulation of VEGFA expression [65]. VEGFA plays a pivotal role in promoting the proliferation of blood vessels, a crucial response aimed at restoring oxygen and energy substrate supply to the retina. However, these newly formed blood vessels often exhibit structural abnormalities that may potentially damage the delicate retinal tissue [66], and in severe cases, this can progress to blindness [9a].

Neurodegenerative eye diseases often manifest with symptoms such as hazy, blurred, or distorted vision. The activation of the APN/AdipoRs signaling pathway has demonstrated significant neuroprotective potential, offering promise for ameliorating these conditions and enhancing visual function. Notably, both APN and AdipoRs are found within various retinal cells [9a]. When APN binds to AdipoRs and subsequently triggers downstream molecular pathways, it exerts its therapeutic effects, with detectable expression in the retina. While adipose tissues predominantly secrete APN [43], it is worth mentioning that the retina [46] and brain [37] can also locally produce this protein. Furthermore, APN readily traverses the bloodstream and efficiently crosses the blood–brain barrier. Numerous pathophysiological disease states, such as elevated blood glucose levels, mitochondrial dysfunctions and dyslipidemia, have the potential to disrupt retinal functions that play a major role in the development of retinal vascular complications [67]. A pivotal driver of glycolysis, specifically isoform 3 of 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase, plays a vital role in the regulation of blood vessel formation [68]. Perturbations in the activity of glucose metabolic enzymes within the polyol pathway can also assume a protective role for the retina, safeguarding it against retinal dysfunction and abnormal blood vessel growth [69,70]. Furthermore, an alternative approach to curbing vessel sprouting involves inhibiting the rate-limiting fatty acid oxidation enzyme, namely, carnitine palmitoyl transferase 1 [71, 9a].

Several studies have explored the protective function of APN/AdipoRs pathways, and one noteworthy investigation was conducted among individuals diagnosed with T2D in Japan. Utilizing laser Doppler velocimetry, the study observed that in males, there was a positive correlation between blood APN levels and retinal blood vessel diameter, as well as retinal blood velocity and flow [72]. However, such a correlation was not observed in females. Achieving a better balance in one's lifestyle or utilizing medications that lead to an elevation in plasma APN levels may unveil a promising avenue for the development of innovative therapeutic strategies in diabetes treatment [72, 9a]. It is worth noting that high glucose levels have been identified as the primary predisposing factor for angiogenesis in DR. Findings from a previous study have elucidated the role of APN in the dysregulated autophagy process and retinal angiogenesis [73]. Additionally, APN has exhibited a protective effect against high glucose-induced damage to RF/6A cells. Furthermore, it has been shown to mitigate high glucose-induced angiogenesis in chorioretinal endothelial RF/6A cells by inhibiting the autophagy pathway [73]. This research

indicates that APN is a promising therapeutic target for treating angiogenesis in diabetic retinopathy (DR). It can effectively reduce retinal neovascularization by inhibiting tube formation in human cell cultures of retinal microvascular ECs, umbilical vein macrovascular ECs, and choroidal ECs [9a]. This effect is associated with the suppression of VEGF's role in DR-related angiogenesis [74,75]. The closely related cytokine, C1q/TNF-related protein-9, can help maintain the blood-retinal barrier (BRB), reducing inflammation in diabetic db/db mice with DR [40]."

4. CURRENT UNDERSTANDING OF THE PATHOPHYSIOLOGICAL ROLE OF APN/AdipoRs IN NEOVASCULAR AMD

Neovascular AMD is one of the major factors in allowed blindness in the elderly. This progressive disease affects the macular region (also called macula lutea) of the eye, a pigmented yellow area mass of the retina, containing color-sensitive rods, which is vital for sharp, central vision. Evidence from the literature suggests a crucial role of APN in ameliorating neovascularization in AMD [8,17, 9a]. Mice with laser-induced neovascularization in the choroid serve as a model to simulate various inflammatory responses associated with AMD [17,18]. The growing body of research indicates that Adiponectin (APN) may emerge as a novel and highly promising therapeutic target for addressing angiogenesis associated with DR. More specifically, it has the potential to significantly reduce the development of new blood vessels in the retina, a process known as retinal neovascularization, in primary human cell cultures of retinal microvascular endothelial cells (ECs), umbilical vein macrovascular ECs, and choroidal ECs. This beneficial effect is closely linked to its ability to interfere with the function of the VEGF, a key driver of angiogenesis in DR [9a].

Importantly, the most closely related counterpart of APN, a cytokine called C1q/TNF-related protein-9, has shown the capability to protect the integrity of the blood–retinal barrier (BRB). Preserving the BRB serves to reduce the inflammatory response observed in diabetic db/db mice affected by DR. In addition to its potential applications in treating DR, therapies centered around APN/AdipoRs may hold promise in addressing the vision-threatening consequences of AMD. AMD encompasses two prevalent phenotypes: dry AMD and wet AMD. This condition is considered by the accumulation of drusen, which are composed of a mixture of proteins, fats, minerals, and other debris, forming spherical structures bound tightly to proteins. With age, drusen inflict damage on the retina, leading to permanent changes in retinal cells [9a]. Dry form can progress to wet form, described by the development of new blood vessels originating from the choroid. These vessels grow in the subretinal space including RPE and, ultimately culminating in central significant vision loss. Mallordo and colleagues have postulated the distinct role of APN in ocular diseases, highlighting its inhibitory effects on the proliferation and migration of RPE cells [76]. Furthermore, Osada and his team demonstrated the consequences of AdipoR1 deletion on abnormal lipid metabolism within the retina, as well as retinal neurodegeneration, using AdipoR1 deleted mice model. Their research, utilizing in situ hybridization, revealed robust AdipoRs mRNA expression in the photoreceptor inner segment (PIS) and faint reactivity in the inner retinal

layers in 4-week-old control mouse retinas [9a]. The expression of AdipoR1 in the retina appears to play a crucial role in inducing the elongase enzyme of very long-chain fatty acids (ELOVL2), a possibly essential step in providing an adequate supply of docosahexaenoic acid (DHA) necessary for the proper functioning and survival of photoreceptor cells [77].

Potentially, the expression of APN and its receptors in single-cell RNA-seq datasets related to AMD can help researchers better understand the possible mechanism of APN/AdipoRs signaling in retinal disease, including its impact on inflammation, angiogenesis, and specific retinal cell populations. This knowledge may ultimately contribute to the development of more effective treatments for AMD [9a]. To date, none of the literature on single-cell RNA-seq presented the expression of APN/AdipoRs in the various retinal cell populations, which emphasizes the need to enumerate the APN pathway genes during AMD pathogenesis. The single-cell RNA-sequencing data for dry AMD were obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo (accessed on 15 August 2023)) under accession number GSE221042. We conducted an in-depth analysis of cellular heterogeneity and landscape using the Seurat package v4.1.1 [78] by plotting UMAP (Uniform Manifold Approximation and Projection). Furthermore, we identified distinct cell types using the corresponding marker genes (Fig. 1A and B) as previously reported by Kuchroo et al. [79, 9a]. By employing marker genes, we were able to distinguish various types of neuronal cells, such as retinal ganglion cells, horizontal cells, bipolar cells, rod photoreceptors, cone photoreceptors, and amacrine cells, in addition to uncovering infrequent non-neuronal cell types, such as microglia, astrocytes, and Müller glia. To understand the cellular expression of APN/AdipoRs and how the AdipoR1/R2 changes during AMD pathogenesis, we plotted feature dot plots (Fig. 1C and D, 9a. Our results demonstrate that expression of both the receptor isoforms (AdipoR1/R2) showed lower expression in dry AMD pathogenesis compared to healthy subjects. Furthermore, we checked the expression of other vascular cell subpopulations. Our analysis revealed the elevation of angiogenesis marker genes VEGFA in Müller glia cells and AMD-associated genes HTRA1 in the horizontal cells during dry AMD pathogenesis [9a]. Dry AMD is characterized by the activation of intrinsic immune cells within the retina, specifically microglia cells, Müller cells, retinal pigment epithelial (RPE) cells, and macrophages. Under dry AMD pathophysiological conditions, Muller cells are involved in retinal angiogenesis [80]. Notably, drusen, a hallmark feature of dry AMD, contains a plethora of pro-inflammatory proteins, including apoE protein, acute phase and coagulation proteins, immunoglobulin G (IgG), complement components, and activators [81]. This indicates that local inflammatory response (ocular) plays an essential role in the early pathophysiology of AMD.

Bushra and colleagues noted that APN played an inhibitory role in the adhesion of endothelial cells (ECs) and the organization of the extracellular matrix. Simultaneously, this led to an enhancement in the barrier function, effectively mitigating the damage induced by high glucose levels in human retinal endothelial cells (HMRECs) [82]. In another study using a diabetic mouse model induced by STZ, researchers examined the impact of APN on the early development of

vascular system damage in the retina. The immunofluorescence findings revealed that APN localized in the vascular endothelium of retinal arterioles in a T-cadherin-dependent manner, which progressively declined as diabetes progressed. This decline in retinal APN expression was concurrent with early signs of DR, characterized by increased vessel permeability. Importantly, treatment with dapagliflozin, a selective inhibitor of sodium–glucose co-transporter 2 aimed at lowering glucose levels, effectively prevented this reduction in retinal APN/AdipoRs system expression [83]. Furthermore, the study found that a deficiency in APN resulted in pronounced vascular permeability during moderately short-term hyperglycemia [9a]. This was accompanied by a significant increase in vascular cellular adhesion molecule-1 (VCAM-1) and a decrease in claudin-5 expression in the endothelium region of the retina [83,84].

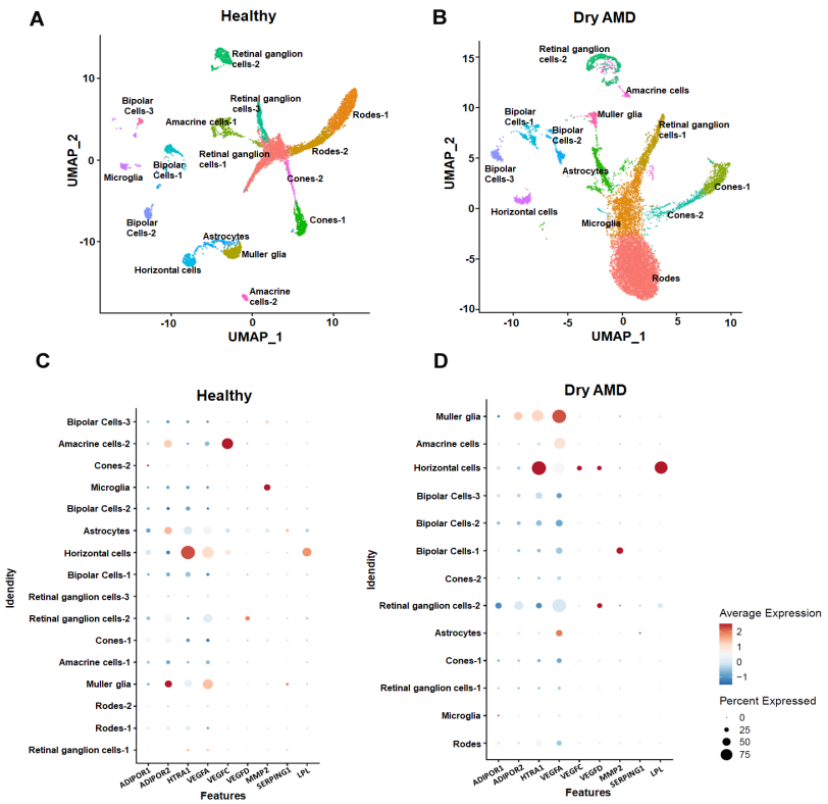

Fig. 1. Single-cell UMAP visualization depicting the cellular landscape of healthy (A) and dry age-related macular degenerated (AMD) eyes (B). The expression of AdipoR1 and AdipoR2 genes in various retinal cell populations of healthy (C) and diseased dry AMD (D). Data were analyzed using the software Seurat v4.1.1 implemented in R v4.2.1

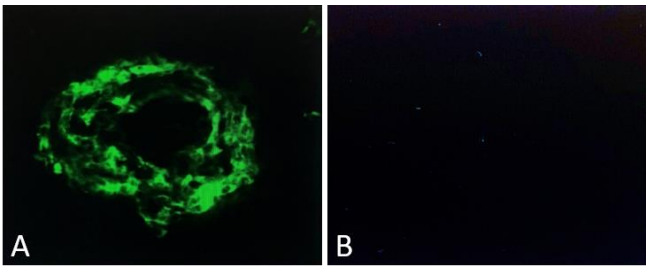

Fig. 2. Laser-induced CNV in mice. Four laser spots were placed in each mouse eye. Animals were sacrificed on day 7 (CNV fully matures on day seven in this model), and confocal pictures were taken after making flat mounts. The green color indicates new vessels formed from choroid (A). Phospho-buffer saline (PBS)-treated mice did not show any green color, indicating no CNV formation from the choroid (B)

Mice deficient in very low-density lipoprotein receptors (Vldlr KO) exhibit pathological angiomatous proliferation in the retina, a disease state that also afflicts people with AMD. A supplementary ingredient ω3 long-chain polyunsaturated fatty-acid (ω3-LCPUFA), added to rodent food in the form of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), is identified to suppress laser-induced CNV in controlled mice whereas this suppression is abolished in APN deficient mice. In addition, in the retinas of Vldlr KO mice, the ω3-LCPUFA can further enhance its receptor1 expression and inhibit CNV. The clinical and experimental evidence suggests that ω3-LCPUFA-rich food may serve a protective role for AMD patients [9a]. In another investigation employing a choroid mice model with laser-induced neovascularization, it was demonstrated that the magnitude of choroidal neovascularization (CNV) could be significantly decreased through the administration of Adiponectin Peptide I (APN1) [15,16,85]. Adiponectin (APN) is recognized for its anti-inflammatory properties and shares over 75% structural similarity with the complement protein C1q. APN1 peptide, derived from the globular domain of APN, exhibited a remarkable inhibition of CNV, surpassing 75% reduction when administered subretinally in comparison to the control peptide [17,18]. CNV was analyzed by confocal microscopy by measuring newly formed green vessels. Newly formed vessels were stained green by perfusion of FITC-Dextran. Image analysis was performed using the ImageJ program (Fig. 2) [9a].

We have designed and synthesized several peptides. APN1, APN2, APN3 and Control peptide. After scanning all the peptides, APN1 inhibited CNV by more than 75%. APN2 and APN3 inhibited CNV by 50% and 40%, respectively. The control peptides did not inhibit CNV at all. Therefore, we used APN1 and Control peptide for our experiments (Fig. 3) [9a]. All control peptides were designed with the same number of amino acids as APN1. We think APN1 may be a better alternative for the most frequently used wet AMD treatments. Anti-VEGF treatments can cause hemorrhage, require frequent injections, and are very expensive for patients.

APN1 is not an anti-VEGF treatment. Rather, it binds to Adiponectin receptor one (AdipoR1), then, through cAMP, it inhibits CNV development [86,87].

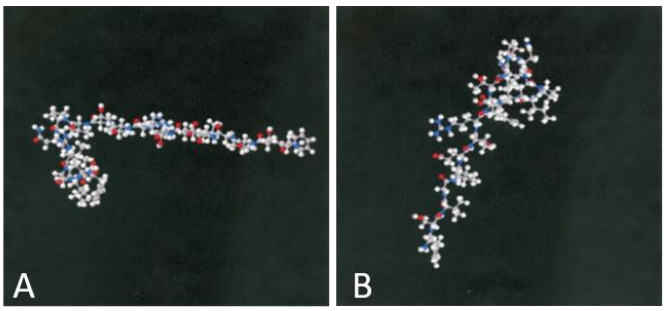

Fig. 3. Three-dimensional (3D) structure of Control peptide. (A) FSVGLETRVTVPNVPIRF and APN1 inhibitory peptide (B) KDKAVLFTYDQYQEKNVD. All modeling calculations were performed using the SYBYL program, and the structure of the peptide was built using the 'build protein' tool in SYBYL. The lowest energy conformer for the peptide was calculated to be 43.145 hartrees [17]

The wet form of AMD is characterized by an excessive growth of new blood vessels around the macula, a region of the retina. This abnormal process is called CNV, which can lead to the development of leaky blood vessels. Therefore, the vision loss associated with wet AMD tends to be more severe compared to the dry form. Recent research indicates that a peptide known as Adiponectin Peptide 1 (APN1) has demonstrated the ability to decelerate the advancement of CNV [9a]. We have designed a peptide APN1 as an agonist and we think, for the purpose of continuous APN1 activity, it can be pegylated for clinically proven effect. Various doses of APN1 can be evaluated in the rodent model to offer clinical insights regarding the optimal dosing for potential human applications. The best dose was 20 µg/Kg, and this will give us some idea about the dose to be used in humans. Once we know the binding mechanism of APN1/AdipoRs, by our experiments or from a literature search, we can design an inhibitor and test it to proceed further to find out whether this inhibitor will also block APN1 binding. Its well established where APN binds to AdipoR1 but not the peptide APN1 that we have designed [28,55,60]. Currently, we are unsure how well APN1 will inhibit wet AMD in humans, but we expect it will be less invasive as an eye drop or may require fewer injections with greater intervals between treatment visits compared to currently available drugs on the market today [9a].

5. EXERCISE, APN/AdipoRs SIGNALING, AND NEOVASCULAR AMD

Physical exercise has the potential to excite the systemic and localized production of APN, offering a protective effect in various ocular conditions, including but not limited to DR, AMD, retinitis pigmentosa (RP), glaucoma, and light-induced retinal degeneration [88]. Engaging in daily physical exercise seems to provide a

safeguard against the development of neovascular AMD. Recent studies observed that AIM2/NLRP2 inflammasome (predominantly expressed in microglia) was most copiously expressed in the retina–choroid complex. They found that the expression of AIM2 was significantly induced during CNV pathogenesis which was significantly attenuated by treadmill training exercise in rodents [88, 9a]. Physical exercise mitigates neovascular AMD by suppressing the AIM2 inflammasome in myeloid cells. Moreover, earlier proteomic data directed that physical exercise facilitated the secretion of APN [9], from fat to circulation, which in turn, led to a decrease in ROS-induced DNA damage and the inhibition of AIM2 inflammasome activation in myeloid cells within eyes affected by choroidal neovascularization (CNV), and this effect was facilitated through the AMPK-p47phox pathway. Findings from earlier studies suggested that in sedentary AdipoQ-deficient (APNKO) mice, there was a tendency for an enhanced leakage area and choroidal neovascularization (CNV) volume when compared to sedentary wild-type mice [88]. Furthermore, this difference became more pronounced in exercised mice, implying that APN plays an essential role in the positive effects observed in exercised mice [89]. Earlier investigation aimed to ascertain if exercise induced an enrichment of APN within the ocular structures. Utilizing Western blotting and ELISA assays, they demonstrated a noteworthy elevation in APN levels within the retina–choroid complex and the vitreous fluid of the exercised mice [90-91]. Notably, while treadmill exercise training significantly reduced the leakage area and CNV volume in wild-type mice, this protective effect was not statistically significant in APNKO mice [88]. These results substantiate the essential role of APN/AdipoRs in mediating the protective benefits of exercise. To obtain more comprehensive results, it is imperative to implement more detailed and rigorous research studies.

6. ADIPONECTIN'S ANTIOXIDATIVE PROPERTIES IN OCULAR TISSUES

Oxidative stress assumes a pivotal role in the pathogenesis of age-associated ocular diseases, notably age-related macular degeneration (AMD), cataracts, and glaucoma. Advancing age is associated with a declining capacity for antioxidant defense mechanisms, leading to an accumulation of reactive oxygen species (ROS) within diverse ocular cell types. This surge in ROS levels precipitates oxidative damage, a hallmark of age-related ocular pathologies [91]. APN hormone has garnered increasing attention for its anti-oxidative properties within the ocular tissues. Emerging research has demonstrated that APN exerts a multifaceted anti-oxidative influence on retinal structures, particularly in the context of aging-related ocular diseases such as AMD and DR. APN appears to modulate oxidative stress by downregulating ROS generation, mitigating lipid peroxidation, and bolstering antioxidant defense mechanisms [9a]. Additionally, APN's capacity to enhance EC function and mitigate inflammation plays a pivotal role in its anti-oxidative effects, as inflammation and oxidative stress are often intertwined in ocular pathologies [92]. The intricate interplay between APN and oxidative stress pathways in retinal cells presents a promising avenue for future investigations and therapeutic interventions aimed at preserving visual health.

7. UNLOCKING CLARITY: ADIPONECTIN'S TRANSFORMATIVE QUEST FROM FAT RESERVES TO OPTICAL RESILIENCE

The multifaceted obesity hormone APN has evolved from being initially perceived as a critical regulator of adipose tissue metabolism to a multifunctional molecule with far-reaching effects across various physiological domains. Beyond its contributions to glucose homeostasis, lipid metabolism, and insulin sensitivity, recent research has unveiled APN's involvement in unexpected domains, such as optical resilience. Intriguingly, APN appear to exert a protective influence on vision, especially retinal function, a feat that illuminates its versatile nature [9a]. This newfound role is rooted in its ability to modulate inflammatory responses, maintain vascular integrity, and mitigate oxidative stress within the intricate microenvironment of the eye. By suppressing the production of inflammatory cytokines, such as TNF-α and interleukin-6 (IL-6), APN helps to create a less hostile environment for retinal cells [83]. Moreover, its vasodilatory properties contribute to improved blood flow, which is crucial for maintaining the highly vascularized retina's health. Additionally, APN's antioxidant effects further shield the delicate retinal structures from oxidative damage. This newfound role in safeguarding the delicate structures of the eye underscores the profound and far-reaching impact of APN on overall health. As we delve deeper into the mechanisms underpinning its actions, the journey of APN from adipose reserves to retinal resilience continues to illuminate novel avenues for therapeutic interventions and a more comprehensive understanding of the intricate interplay between metabolic and physiological processes [9a].

8. FUTURE DIRECTIONS FOR RESEARCH AND CLINICAL APPLICATIONS

The multifaceted benefits of APN/AdipoRs signaling have been demonstrated across various cell types, encompassing insulin-sensitizing actions, anti-inflammatory effects, anti-atherosclerotic properties, anticarcinogenic potential, and antiproliferative activities. Given APN's ameliorative role in mitigating insulin resistance, diabetes, and age-related conditions, a decline in APN levels is deemed pivotal in the pathophysiology of retinal diseases. It is also associated with the susceptibility to diabetes-related diabetic retinopathy (DR) and age-related macular degeneration (AMD)-related neovascularization [9a]. The investigation of the critical roles of AdipoR1 and AdipoR2 has gained momentum following the cloning of these two adiponectin receptors, which confirmed their essential role in APN binding and its subsequent glucose-lowering effects. Moreover, APN activation can trigger the activation of AMPK/SIRT1/PGC-1α and nuclear receptors PPARs through the AdipoRs signaling pathway. The screening of low molecular weight compounds to identify AdipoR1/R2 agonists, along with various therapeutic approaches, offers the potential to develop novel therapeutic strategies. Utilizing 3D conformational analysis of AdipoRs, optimization of AdipoRs agonists can be achieved, enabling the development of effective, safe, and high-quality drugs for treating debilitating eye conditions. Future research endeavors should prioritize elucidating the functions of AdipoR1 and AdipoR2 and targeting their agonists to create innovative anti-aging and anti-diabetic pharmaceuticals [9a]. This approach

not only enhances our understanding of the molecular mechanisms underlying APN's activity but also contributes to addressing obesity-related and other metabolic disorders.

Moreover, age-related macular degeneration (AMD), diabetic retinopathy (DR), and retinopathy of prematurity have all been linked to disruptions in the circulating function of APN/AdipoRs or alterations in the distribution of APN variants. In experimental settings, APN has shown its potential to counteract retinal defects and choroidal neovascularization (CNV). Given its pivotal role as a regulator of glucose and lipid metabolism, APN-derived peptides hold promise in restoring metabolic equilibrium [9a]. Interventions involving ω3 long-chain polyunsaturated fatty acids (LCPUFA) and fibric acid derivatives have been shown to boost APN levels in the bloodstream. Physical exercise has the capacity to stimulate systemic and local APN production, contributing to its protective effects in various ocular diseases, including DR, AMD, retinitis pigmentosa (RP), glaucoma, and light-induced retinal degeneration. Further planned investigations are necessary to deepen our understanding and elucidate the role of APN/AdipoRs in neovascular AMD. Additionally, research efforts should aim to uncover the underlying molecular mechanisms, thus advancing our comprehension of both the experimental and clinical implications of this pathway [9a].

9. CONCLUSIONS

In conclusion, “From Fat to Sight” provides a captivating journey into the enthralling world of APN/AdipoR1/2, intertwining the realms of adipose biology and ophthalmology. This innovative review not only piques the curiosity of researchers but also holds promise in revolutionizing future approaches to combating aging-associated eye disorders [9a]. In this comprehensive review, we aim to provide a deeper understanding of the intricate relationship between adiponectin and eye disorders associated with aging. By elucidating the molecular mechanisms, clinical associations, and therapeutic implications, we hope to inspire further research and innovative strategies for the prevention, diagnosis, and treatment of ocular diseases. The remarkable connection between fat, in the form of adiponectin, and sight opens exciting avenues for advancing eye health and improving patient outcomes. Our future plans are to conduct clinical studies using APN1 and control peptides, injecting both in human vitreous separately to see if this peptide [APN1] can be used for the treatment of human AMD compared to control peptide because we know that APN1 inhibits CNV and leakage in animal models. Multifarious beneficial effects of APN/AdipoRs signaling have been exerted in numerous cell types, such as insulin-sensitizing, anti-inflammatory actions, anti-atherosclerotic, anti-carcinogenic, and antiproliferative effects. Since APN has an ameliorating function on insulin resistance, diabetes, and aging, a reduced APN level is considered to play a vital role in the pathophysiology of retinal diseases and is associated with the possibility of developing diabetes-associated DR and AMD-associated neovascularization. The research to prove the significant roles of AdipoR1 and AdipoR2 gained momentum due to the cloning of these two adiponectin receptors, confirming their requisition for binding of APN and subsequently its glucose-lowering effect. Future research should focus on

clarifying AdipoR1/2 and targeting its agonist to develop novel anti-aging and anti-diabetic drugs, all while facilitating both the concept of molecular mechanisms of APN activity and obesity-related and other metabolic disorders.

Furthermore, AMD, DR, and retinopathy of prematurity are all associated with altered circulating APN/AdipoRs function or APN variant distributions. Experimentally, APN inhibits retinal and CNV defects. As a key glucose and lipid modulator, APNs may re-establish metabolic balance. Intervention with ω-3 LCPUFA and derivatives of fibric acid enhances levels of APN in the blood. Exercise may exert a positive production of APN systemically as well as locally and it plays a protective role in several eye diseases, such as DR, AMD, RP, glaucoma, and light-induced retinal degeneration. Additional planned studies are needed to further investigate and clarify the role of APN/AdipoRs in neovascular AMD as well as the underlying molecular mechanisms to better understand both the experimental and clinical impact of this pathway [93-95].

In this comprehensive review, we aim to provide a deeper understanding of the intricate relationship between adiponectin and aged eye disorders. By elucidating the molecular mechanisms, clinical associations, and therapeutic implications, we hope to inspire further research and innovative strategies for the prevention, diagnosis, and treatment of ocular diseases. The remarkable connection between fat, in the form of adiponectin, and t654r4dexzsight opens exciting avenues for advancing eye health and improving patient outcomes.

INSTITUTIONAL REVIEW BOARD STATEMENT

The Institutional Animal Care and Use Committee (IACUC) at the University of Arkansas approved this study for Medical Sciences, Little Rock, AR. ID Puran Bora 4008. No human subjects were used in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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MDCT in Management of Ovarian Masses

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ABSTRACT

Ovarian tumors are epithelial, mesenchymal, mixed epithelial and mesenchymal, sex stromal and germ cell tumors. Serous and mucinous cystadenoma and adenocarcinoma are differentiated by larger solid component, papillary projections and thick enhancing septae. Serous cyst adenomas and carcinomas are usually bilateral and have more papillary projections as opposed to mucinous neoplasms (usually unilateral and papillary projections are less marked). CT is the modality for staging. Adjacent pelvic organ involvement may be difficult to diagnose accurately. In a large ovarian tumour, it may be difficult to identify uterus which is partially or completely surrounded by tumour. Focal obliteration of fat plane or tumour encasement of bladder or recto-sigmoid is highly suspicious of involvement of the structures Pelvic side wall invasion is suspected when tumour lies within 3mm of pelvic side wall or when iliac vessel is surrounded or displaced by tumour. Teratomas are having solid, cystic, fat component and calcification. Brenner's tumor is detected incidentally. Sex cord sclerosing stromal tumors are unilateral and may be hormonally active. Krukenberg metastases are solid and bilateral.

Keywords: Papillary projections; adenocarcinomas; ovarian cancers; ultrasonography.

1. INTRODUCTION

Ovarian cancers are the third most common gynecological cancers globally in 2020 [1]. According to World Health Organization (WHO), 2020, the ovarian tumors include as epithelial, mesenchymal, mixed epithelial, mesenchymal, sex stromal tumour, germ cell tumors and tumor-like lesions [2]. Ultrasonography with color Doppler is the choice of imaging for the initial evaluation of any suspected adnexal mass. Transvaginal sonography is better than trans-abdominal sonography. However, Computed Axial Tomography (CT) is more suited for

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diagnosis and staging of tumor. Local extent and deposits on the peritoneum, liver, mesentery, and bowel are well demonstrated by CT. Prediction of resectability can be determined by CT [3]. In this chapter, the imaging features of some important ovarian masses by MDCT are described pictorially and relevant comments are made [3a].

Classification of ovarian tumors (WHO 2020): The ovarian tumors are divided into three major categories, which are named according to their presumed histogenesis and direction of differentiation: 1. Common epithelial tumors; 2. Sex cord-stromal tumors and 3. Germ cell tumors [4, 3a]. A minority of ovarian tumors are classified separately either because their histogenesis is uncertain, their cellular components are of several origins, or they are nonspecific tumors that also occur at other sites. A final category of lesions that merit consideration in a discussion of ovarian tumors is various non-neoplastic disorders that simulate neoplasms on gross and sometimes on microscopic examination. The World Health Organization Histological Classification of Ovarian Tumors published in 2020 is presented in Appendix 1 [3a].

Solid ovarian neoplasms account for a minority of ovarian neoplasms. They comprise of a wide pathological spectrum which includes epithelial tumour (28%), Brenner tumor, germ cell tumors, ovarian teratoma- non cystic type (22%), sex cord stromal tumour, ovarian fibroma, ovarian fibro-thecoma, ovarian thecoma and metastatic tumors (20%- including Krukenberg tumour) [5,6].

2. EPITHELIAL TUMORS

They may be benign or malignant. Benign epithelial tumour has fewer papillary projections than malignant. Large papillary projections and irregular solid components suggest malignancy [3a]. On CT, benign epithelial tumors are either mucinous or serous cystadenomas appear as a thin-walled cystic lesion with a soft tissue component, smooth thin wall and papillary projections (Figs. 1, 2) [7,8].

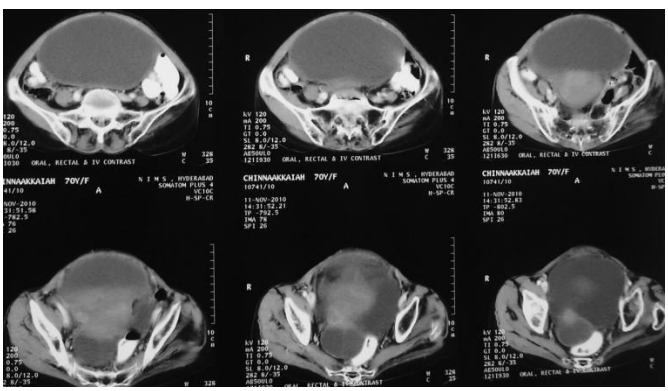

Fig. 1. 70 female: Bilateral benign serous cystadenoma: thin wall, no solid component [3a]

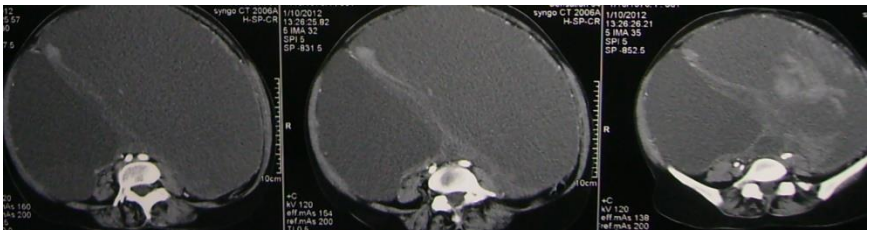

Fig. 2. 36 female: CECT pelvis showing bilateral serous cystadenoma: large cystic space occupying lesion with small solid component [3a]

Malignant tumors have a more complex appearance. They may be unilateral or bilateral solid cystic masses [3a]. Multiloculated appearance with thick irregular enhancing septations is common (Fig. 3). Papillary excrescences may be noted (Fig. 4).

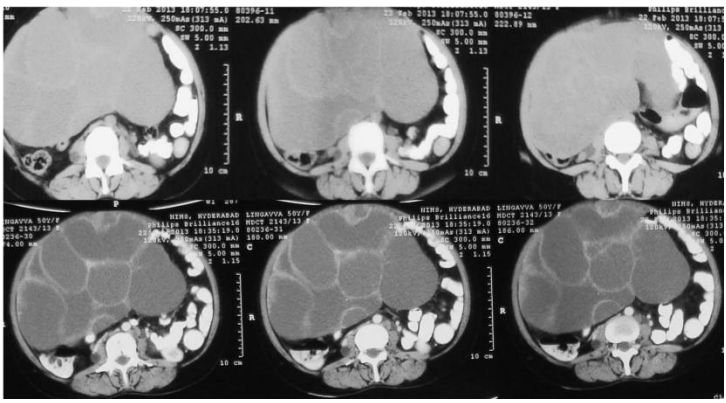

Fig. 3. 50 female: large cystic mass with thick enhancing septae in a case of serous cystadenocarcinoma [3a]

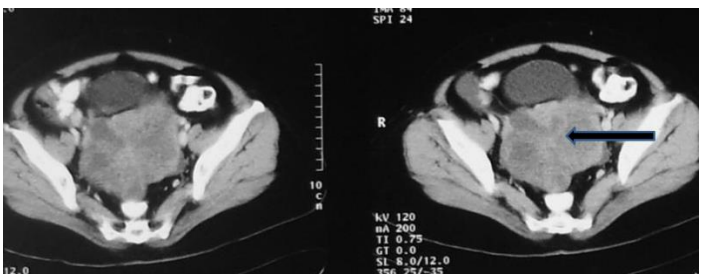

Fig. 4. 60/female: serous cystadenocarcinoma ovary (arrow points to thick septae)

Serous cystic neoplasms may be low grade (LGSC) or high-grade tumour (Serous cystadenocarcinoma). These borderline or low-grade lesions have various solid components including papillary projections or mural nodules. Rarely do they have surface nodules. On contrast administration these solid components enhance. These borderline tumors are bilateral in one-third of cases and the rest are low grade type [9]. High grade serous cystadenocarcinomas present as cystic masses with variable solid components. Papillary projections are common, and they demonstrate intense enhancement. In up to 58% of cases the tumors are bilateral. In some cases, solid components may be of high attenuation representing psammoma calcification.

Benign mucinous tumour affects young females, and they have good prognosis. The malignant mucinous tumors have worse prognosis. These tumors are unilateral multiloculated with variable signal intensity, attenuation and echogenicity of mucin content cysts. This is due to the different viscosity of mucin. Papillary projections seen in serous cystic tumour are not usually observed. Malignant mucinous neoplasms are large (>10 cm) with significant solid component (Fig. 5). Small multiple locules may be seen giving honeycomb appearance.

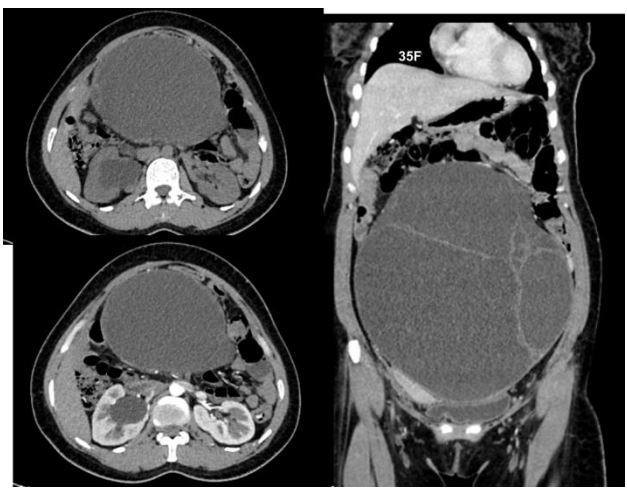

Fig. 5. A large mucinous adenocarcinoma in 35years female [3a]

Defining the extent of disease or staging: Imaging is done prior to laparotomy which is the gold standard. CT imaging helps to plan surgery and to decide optimal de-bulking [3a]. In general CT is preferable to MR as it is readily available and quicker. Imaging features of CT and MR are similar and can detect involvement of pelvic and abdominal structures. Staging criteria for CT and MRI have been adapted from the International Federation of Gynecology and Obstetrics (FIGO) classification system of ovarian cancer [3a, 10].

Stage I: Confined to one ovary is stage 1a; if both sides- 1b- capsule of tumour is intact and there is no evidence of tumour spread to ovarian surface; 1c tumour spread to ovarian surface or capsule ruptured (Fig. 6) or malignant cells in ascites or peritoneal washings [3a].

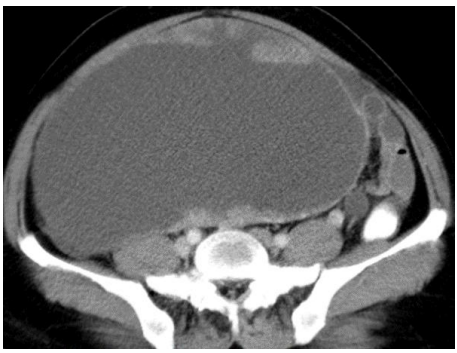

Fig. 6. Stage I: Carcinoma ovary with intact capsule and smooth outer surface [3a]

IC-1: Surgical Spill
IC-2: Capsule rupture before surgery
IC -3: Malignant cells in the ascites or peritoneal washing

Stage II: Tumour extends to pelvic soft tissues, or organs in pelvis.

In stage 2a: extension to uterus and/ or fallopian tube.

2b: extension to other pelvic organs such as bladder, rectum, peritoneum. Bowel or bladder involvement is suggested by loss of fat plane between the organ and mass, encasement or localized thickening [3a]. A distance of 3mm between mass and muscle of pelvic side wall or displacement or encasement of iliac vessel is highly suggestive of pelvic side wall invasion.

Stage 2c- Features of 2a or 2b plus pelvic ascites (Fig. 7)

Stage III: Peritoneal implants outside pelvis and retroperitoneal lymphadenopathy. Implants can be omentum, liver, parietal peritoneum. Peritoneal dissemination is characterized by peritoneal thickening, nodular lesion, stellate nodules located within mesentery or omentum [3a]. Stage 3 a, b, c differs in size of lesion- 3a-tumour grossly limited to pelvis and gross ascites; 3b-peritoneal implant 2cms or less; 3c- implant size is more than 2cms. Retroperitoneal adenopathy qualifies as stage 3c (Figs. 8, 9, 10).

Stage III A1: positive retroperitoneal lymph nodes only
A1-i: Mets up to 10 mm in greatest dimension
A1-ii: Mets > 10 mm in greatest dimension

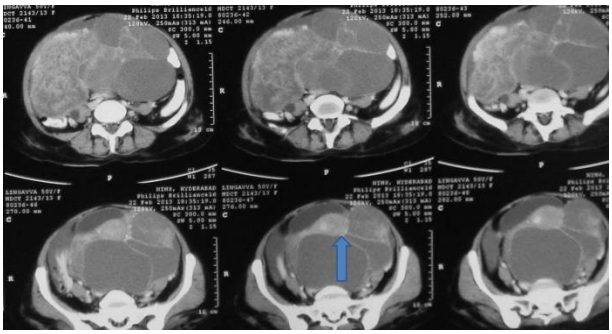

Fig. 7. Papillary serous cystadenocarcinoma ovary in 50 female. Large solid enhancing component, thick septae, encasing uterus indicated by arrow with minimal ascites (Stage II) [3a]

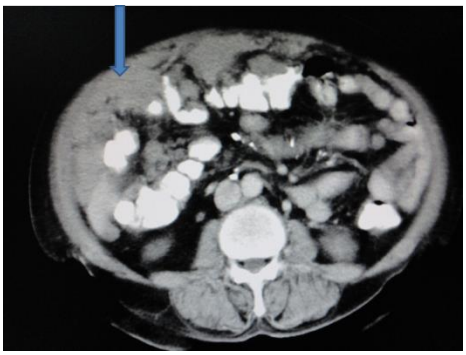

Fig. 8. 52 female: Omental deposits (arrow): Stage III disease [3a]

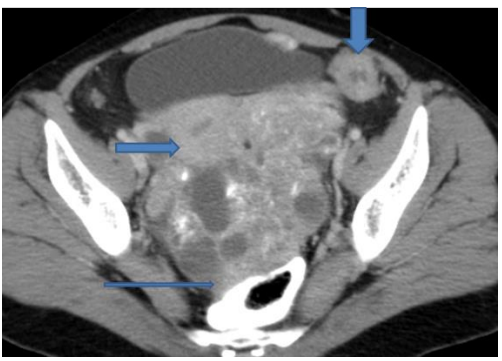

Fig. 9. 24 female: mucinous carcinoma ovary, thick septation, infiltrating uterus, rectum and pelvic side wall and peritoneal deposit (arrow) [3a]

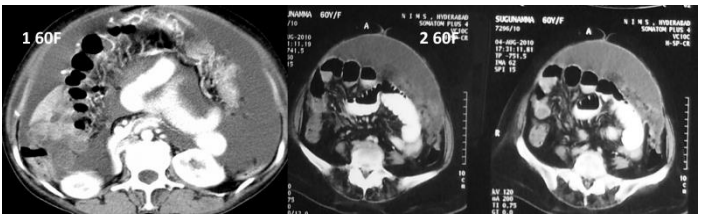

Fig. 10. Two different cases of carcinoma ovary with omental and peritoneal thickening [Stage III] [3a]

Stage III A2: Microscopic peritoneal metastasis beyond pelvis < 2 cm in greatest dimension with or without metastasis to retroperitoneal lymph nodes.

Stage III C: Macroscopic peritoneal metastasis beyond the pelvis > 2 cm in greatest dimension with or without metastasis to retroperitoneal lymph node.

Stage IV: Distant metastases, pleural effusion, pleural nodules or focal thickening suggest this stage (Figs. 11, 12).

Accuracy for detecting peritoneal deposits is dependent on their location, size and presence of ascites. MRI and CT have similar sensitivity in detection of peritoneal deposits greater than 1cm. Peritoneal deposits appear as rounded, cake-like, stellate, or ill-defined masses [3a]. However, deposits in mesentery/ implant on surface of bowel and calcified deposits are better seen in CT. Adjacent pelvic organ involvement may be difficult to diagnose accurately. In a large ovarian tumour, it may be difficult to identify uterus which is partially or completely surrounded by tumour. Focal obliteration of fat plane or tumour encasement of bladder or recto sigmoid is highly suspicious of involvement of the structures. Pelvic side wall invasion is suspected when tumour lies within 3mm of pelvic side wall or when iliac vessel is surrounded or displaced by tumour. Omental cake represents replacement of normal fat of omentum by a soft tissue density and the causes include peritoneal metastasis from carcinoma of colon, ovary, pancreas, stomach, and breast and from lymphoma, mesothelioma, and tuberculosis of the peritoneum (Fig. 12). Staging accuracy is 80-90% [11, 3a].

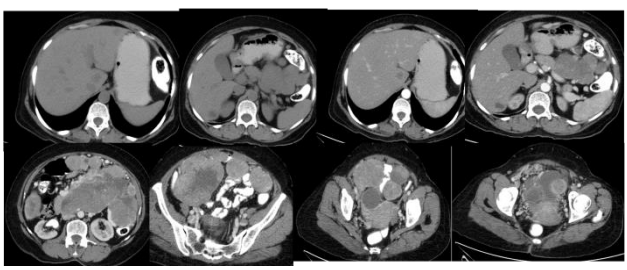

Fig. 11. 67 female: High grade serous cystadenocarcinoma ovary with peritoneal and liver metastases (Stage 4) [3a]

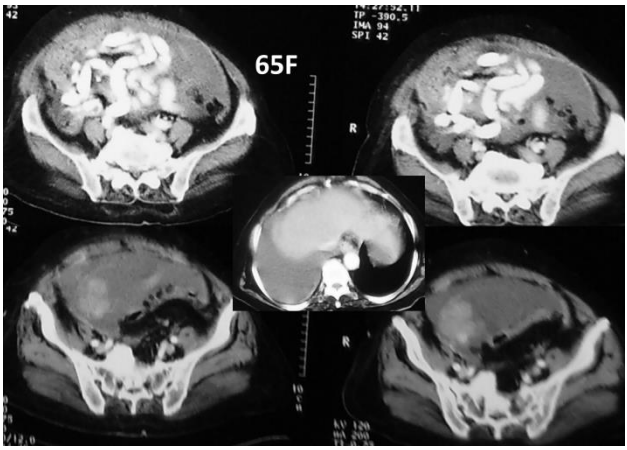

Fig. 12. Stage IV disease in 65 female: there is pleural effusion, ascites, omental deposits forming omental cake [3a]

Stage IV: Distant metastasis excluding peritoneal metastasis.

IV-A: Pleural effusion with positive cytology
IV-B: Parenchymal metastasis and, metastasis to extra-abdominal organs (including inguinal lymph nodes, lymph nodes outside abdomen and pelvis).

Germ cell tumour: Ovarian cystic teratoma contains mature epithelial elements such as sebum, hair, epithelium, calcium, desquamated skin, and other elements which give complex appearance. Although they do not contain fat, they contain sebum which is lipid material with characteristic signal similar to fat. This differentiates it from other masses (Figs. 13, 14). Malignancy associated with mature cystic teratoma is rare and occurs in 1-2% of cases. Malignant transformation is seen in tumour larger than 10 cms [3a]. Tumour presents with large solid and little fat component with enhancing irregularly margins of solid component. Solid component tends to show extensive trans mural invasion and extension to adjacent structure (Figs. 15, 16). Enhancement of Rokitansky protuberance should raise the possibility of malignant transformation. Rarely can it appear as large cystic lesion containing fat fluid level. When there is Scanty or punctate areas of fat, calcification in a large cystic mass in a child or young adolescent are suggestive of immature teratoma as opposed to coarse or tooth like calcification in mature teratoma (Fig. 17). Mixed germ cell tumour also reveals similar imaging features (Fig. 18A). Elevated serum Alfa-feta-protein, HCG; younger age group can help in differentiating the two (Fig. 18B).

Brener tumour: These rare epithelial tumors occurred in the fifth decade. They are incidental findings in most of cases and are smaller than two cms. Occasionally the tumor may be >10cm having solid and cystic component. On imaging, they are unilateral solid mass showing amorphous calcification. Solid

components show mild to moderate enhancement. Lack of local invasion, peritoneal metastases, and ascites differentiate these tumors from other (Fig. 19).

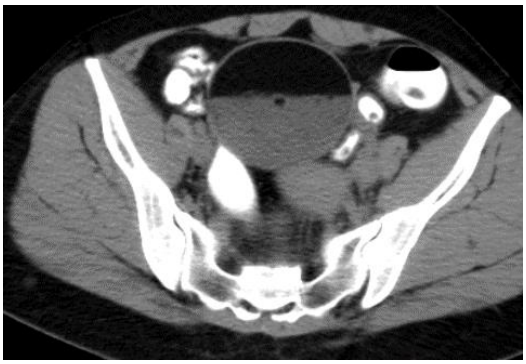

Fig. 13. 38 female: Ovarian dermoid showing calcification and fat- fluid level [3a]

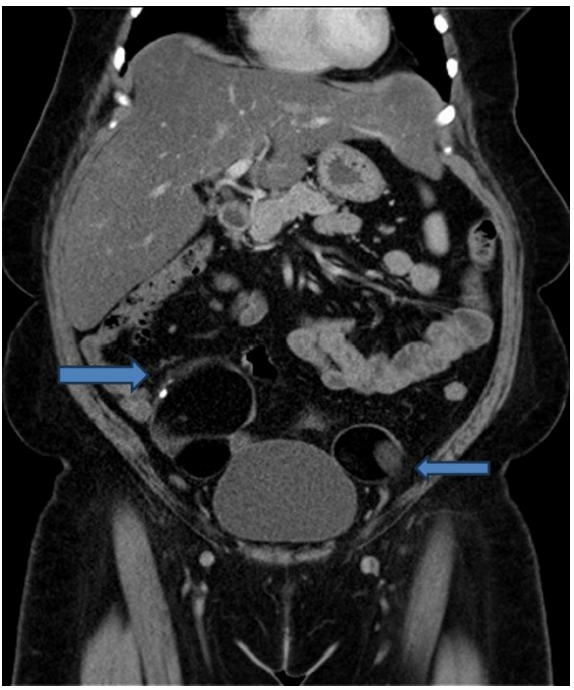

Fig. 14. 45 female: Bilateral ovarian dermoid [3a]

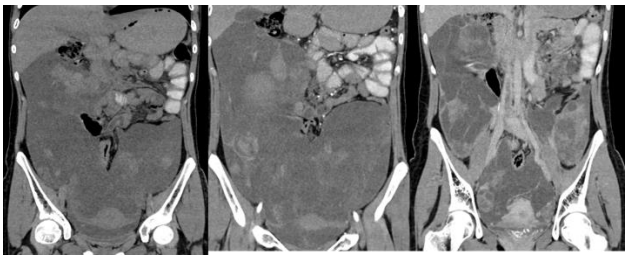

Fig. 15. 17 female: immature teratoma -large cystic mass with calcifications and enhancing large solid component with invasion to adjacent structures and peritoneum [3a]

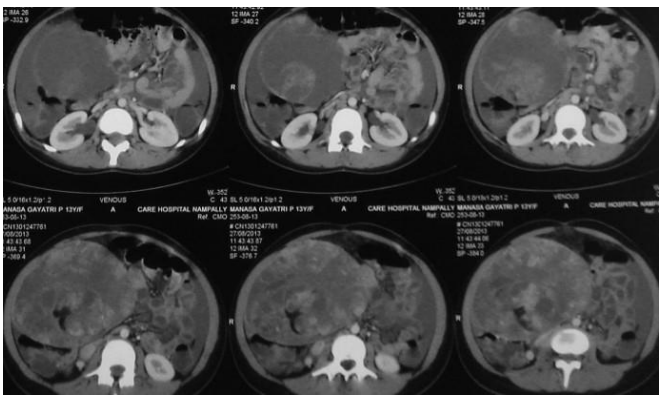

Fig. 16. Immature teratoma in 13 female: Size is more than 10 cms having fat, large solid enhancing component. There is ascites. [3a]

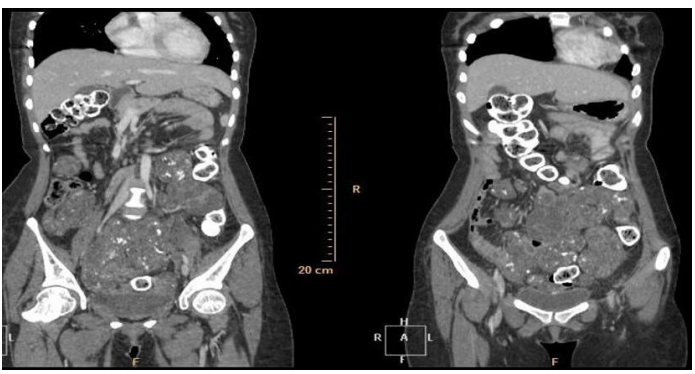

Fig. 17. 20 female: Immature teratoma showing punctate calcification and large soft tissue mass with extension to peritoneum and mesentery [3a]

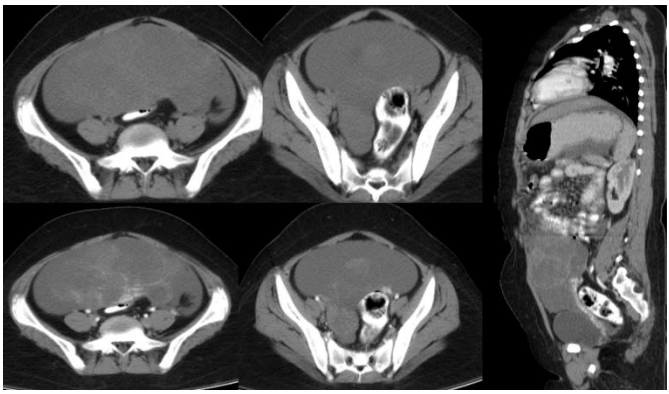

Fig. 18A. Mixed germ cell tumor with elevated alfa- fetoprotein and beta HCG: Large solid cystic mass with calcification [3a]

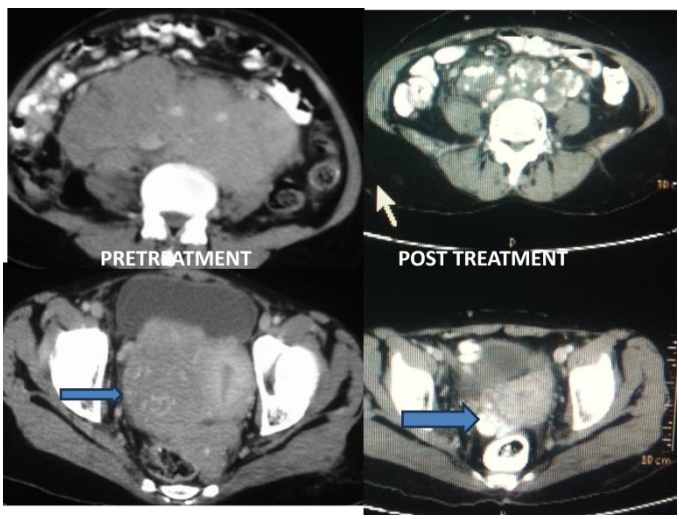

Fig. 18B. 31female: Germ cell tumor right ovary: pre and post chemotherapy there is significant reduction in size of ovarian mass with dense calcification indicated by arrow. Retroperitoneal adenopathy also shows significant resolution with calcification. Note pretreatment ovarian mass was infiltrating uterus, bladder and rectum. Lymph nodes were also encasing aorta and IVC [3a]

Dysgerminoma: These are malignant solid masses which have cystic solid areas, necrosis and hemorrhage occurring in young adults/adolescents. Predominantly solid encapsulated with characteristic fibrovascular septae. They show homogenous enhancement [12, 3a].

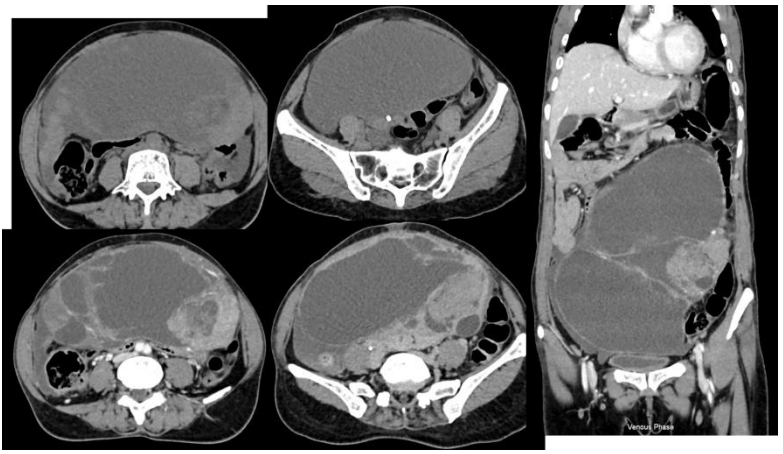

Fig. 19. Brenner's tumor -large solid cystic mass without invasion, ascites, peritoneal deposits. Note a focus of calcification in it [3a]

Sex cord Sclerosing stromal tumour: These arise from stromal cells and primitive sex cord in ovary. Common types are granulosa cell tumors, fibrothecomas, sclerosing stromal tumors and Sertoli-Leydig cell tumour. These tumors affect all ages. They are present commonly in stage 1, itself as tumors are hormonally active. Treatment is often surgical.

Ovarian fibroma and fibrothecoma: These are benign tumors of stromal origin and constitute 3-4% of all ovarian malignancy. Typically, they are unilateral in 90% and occur in peri and postmenopausal women. Fibroma is the most common sex-cord tumour. It can be associated with Meig's syndrome (ascites, ovarian tumour and right sided pleural effusion). Fibrosarcomas are rare. They are homogeneous solid masses with delayed enhancement on CT. On MRI, hypointense on T1 and T2 weighted images. Dense calcification may be seen. Scattered areas of hyper intensity may represent oedema /cystic component. Torsion is a common complication.

Sclerosing Stromal Tumour: Young female patients present with vaginal bleeding. Imaging shows large mass with cystic component or heterogenous solid mass of intermediate to high signal on T2 weighted MRI. Malignant sex cord tumor and granulosa cell tumour are two types-juvenile and adult type. Juvenile type present before puberty and may present as pseudo puberty. They have an excellent prognosis. Adult type constitutes 90% presenting with abnormal uterine bleeding. Granulosa cell tumour has a tendency for hemoperitoneum. Size is variable. Morphology is variable may be cystic to complexly solid. The heterogenicity of the mass is due to fibrotic and hemorrhagic components. Sometimes Granulosa cell tumour may present with multilocular cystic appearance due to macro-follicular pattern Thick hypointense rims on T2 represent compressed ovarian stroma. On dynamic contrast scan reveals early

peripheral enhancement with contrast progression. The early peripheral enhancement represents an area of high vascular network. And central delayed enhancing portion represents collagenous hypocellular areas. They are associated with endometrial abnormality-endometrial hyperplasia, polyp carcinoma is common [13] (Figs. 20, 21).

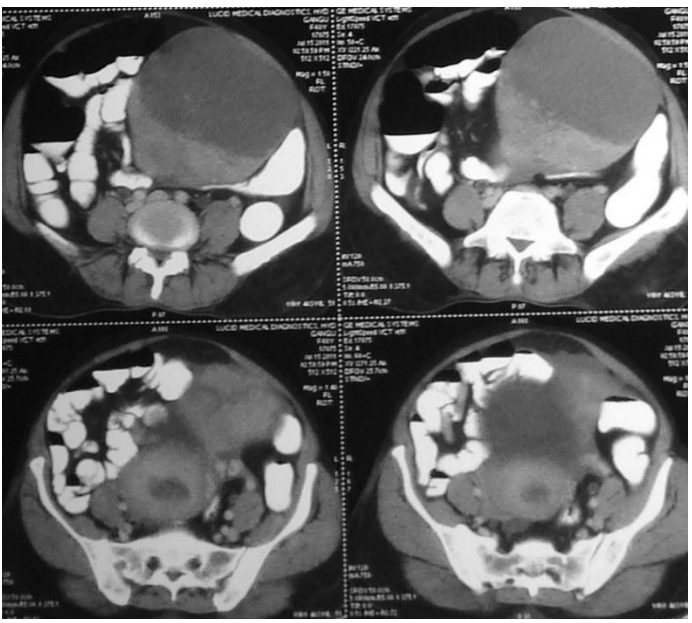

Fig. 20. Granulosa cell tumor showing enhancing solid cystic mass with thickened endometrium [3a]

Sertoli-Leydig cell tumor occurs in younger age and tends to be unilateral. Size is variable; may appear as solid/ solid with peripheral cyst/ cystic lesion with solid mural component or completely cystic [3a]. Well defined enhancing solid tumour with variable intra tumoral cystic component (Fig. 22). T2 hypo-intensity in solid component represents extent of fibrous stroma.

Ovarian metastases: Metastases constitute about 5-15% of ovarian masses. The stomach, colon, breast, pancreas and lung are the most common primary sites. Krukenberg tumors are ovarian metastases with mucus filled signet ring cells. They display bilateral, oval/ lobulated solid or predominantly solid with central necrosis (Fig. 23). On CT/ MR they show sharp contrast enhancement. Tumors can spread by direct invasion, trans-coelomic dissemination, and hematogenous route. Non- Krukenberg metastases appear like primary ovarian malignancy. They are usually bilateral and may be solid and cystic or complex lesion; may be multilocular and associated with ascites and difficult to differentiate from primary ovarian malignancy [13].

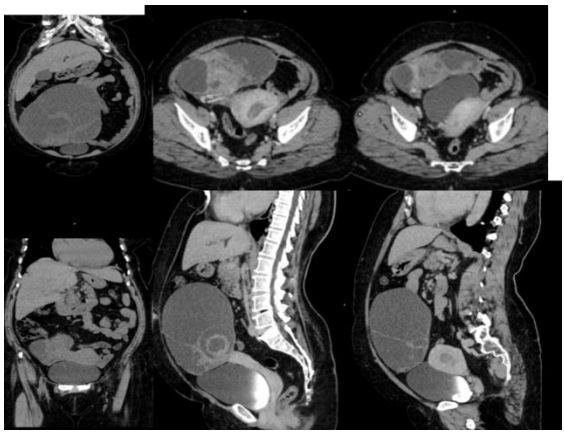

Fig. 21. Granulosa cell tumor in a young female reveals large solid cystic mass and thickened endometrium [3a]

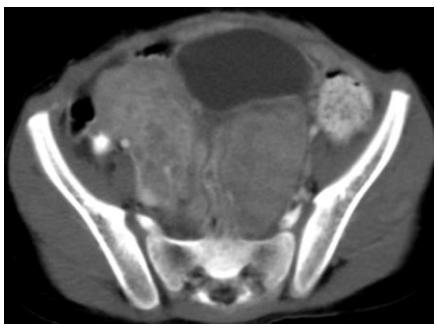

Fig. 22. 7 female: Sertoli cell tumors in both ovaries. The tumor is solid with heterogeneous enhancement [3a]

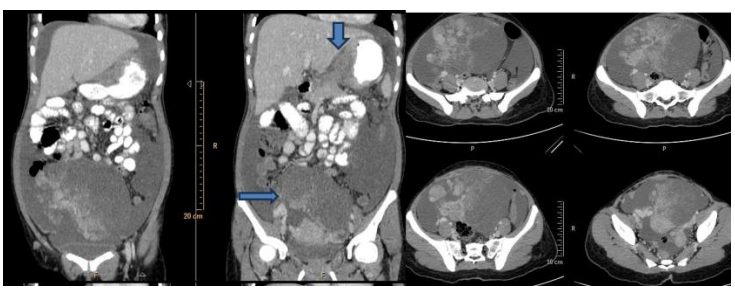

Fig. 23. Krukenberg tumor - mucinous adenocarcinoma of stomach with spread to bilateral ovaries (solid cystic masses) indicated by arrow in 23/female [3a]

3. CONCLUSIONS

Ovarian masses include both benign and malignant tumors. Imaging can differentiate benign from borderline or malignant cases. CT plays a major role in early diagnosis, staging and in management of malignant ovarian neoplasms.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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APPENDIX 1

2020 WHO -Histological Classification of Ovarian Tumors

I***. Epithelial Tumors***

A. Serous tumors

- Serous cystadenoma, adenofibroma and surface papilloma
- Serous borderline tumor

B. Serous borderline tumor, micropapillary variant

- Low grade serous carcinoma
- High grade serous carcinoma

C. Mucinous tumors

- Mucinous cystadenoma and adenofibroma
- Mucinous borderline tumor
- Mucinous carcinoma

D. Endometrioid tumors

- Endometrioid cystadenoma and adenofibroma
- Endometrioid borderline tumor
- Endometrioid carcinoma
  - Clear cell tumors
- Clear cell cystadenoma and adenofibroma
- Clear cell borderline tumor
- Clear cell carcinoma
  - Seromucinous tumors
- Seromucinous cystadenoma and adenofibroma
- Seromucinous borderline tumor
  - Brenner tumors
- Brenner tumor
- Borderline Brenner tumor
- Malignant Brenner tumor
  - Other carcinomas
- Mesonephric-like adenocarcinoma
- Undifferentiated and dedifferentiated carcinoma
- Carcinosarcoma
- Mixed carcinoma

II. *Mesenchymal tumors*

- Endometrioid stromal sarcoma

- Low grade
- High grade

- Smooth muscle tumors
  - Leiomyoma
  - Smooth muscle tumor of uncertain malignant potential
  - Leiomyosarcoma
- Ovarian myxoma

III. *Mixed epithelial and mesenchymal tumors*

- Adenosarcoma

IV. *Sex cord stromal tumors*

- Pure stromal tumors
  - Fibroma, NOS
  - Cellular fibroma
  - Thecoma
  - Luteinized thecoma associated with sclerosing peritonitis.
  - Sclerosing stromal tumor
  - Microcystic stromal tumor
  - Signet ring stromal tumor
  - Leydig cell tumor
  - Steroid cell tumor, NOS
  - Malignant steroid cell tumor
  - Fibrosarcoma
- Pure sex cord tumors
  - adult granulosa cell tumor
  - Juvenile granulosa cell tumor
  - Sertoli cell tumor, NOS
  - Sex cord tumor with annular tubules
- Mixed sex cord stromal tumors
  - Sertoli-Leydig cell tumor
  - Well differentiated.
  - Moderately differentiated.
  - Poorly differentiated
  - Retiform
  - Sex cord stromal tumor, NOS
  - Gynandroblastoma

V*. Germ cell tumors*

- Teratoma, benign
- Immature teratoma, NOS
- Dysgerminoma
- Yolk sac tumor
- Embryonal carcinoma
- Choriocarcinoma, NOS

- Mixed germ cell tumor
- Monodermal teratomas and somatic type tumors arising from a dermoid cyst
  - Struma ovarii, NOS
  - Struma ovarii, malignant
  - Strumal carcinoid
  - Teratoma with malignant transformation
  - Cystic teratoma, NOS
- Germ cell sex cord stromal tumors
  - Gonadoblastoma
  - Dissecting Gonadoblastomaa
  - Undifferentiated gonadal tissue
  - Mixed germ cell - sex cord stromal tumor, unclassified

VI. *Miscellaneous tumors*

- Rete cystadenoma, adenoma and adenocarcinoma
- Wolffian tumor
- Solid pseudopapillary tumor
- Small cell carcinoma of the ovary, hypercalcemic type
- Wilms tumor

VII*. Tumor-like lesions*

- Follicle cyst
- Corpus luteum cyst
- Large solitary luteinized follicle cyst
- Hyperreactio luteinalis
- Pregnancy luteoma
- Stromal hyperplasia and hyperthecosis
- Fibromatosis and massive edema
- Leydig cell hyperplasia

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A Case Report of Missed Femur Fractures in Pediatric Trauma Patient

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ABSTRACT

Isolated paediatric non-displaced femoral fracture is common and can be missed due to many factors. These factors might be related to patient, clinical, technical and radiological misinterpretations. Careful listening to patient complaints and full physical examination is very important in every pediatric trauma. A careful and systematic approach in radiological evaluation by both treating orthopaedic surgeons and radiologists is an important factor in reducing missing a hidden fracture. In polytrauma, children always do a systematic and complete physical examination of the skeletal system. In suspected cases of unseen fractures, further studies might be indicated like bone scan or MRI. Never let a child go home if he/she can not walk or is having difficulty walking without a close follow-up. In this section, we discuss the non-displaced fracture of the femur in children and how to avoid missing a fracture.

Keywords: Missed; missing; fracture; fracture femur; fracture in children; pediatric femur fracture; missed injury; un-displaced fracture.

1. INTRODUCTION

Femoral shaft fractures in children are common [1]. Isolated pediatric femoral shaft fractures occur at an annual rate of 19 per 100,000 and represent the most frequent pediatric orthopaedic injury requiring hospitalization [2,3]. The treatment of these fractures is dictated by various factors. They can be treated with either non-operative or operative procedures. Non-operative methods include Pavlik harness, early spica casting, and traction [4,5]. Femoral shaft fractures are among the most common fractures requiring acute orthopedic intervention and

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hospitalization in children [6]. The orthopaedic surgeon caring for these fractures must take into account the patient's age, weight, family circumstances, and fracture pattern to determine the best treatment options [7]. The cost of these various treatment options can also come into consideration [2,3,8].

A recent annual review of paediatric trauma patients from our institution found five patients (1.6%) with missed fractures and other musculoskeletal (MSK) injuries [9,10]. Several previous studies have noted a high incidence of missed or occult MSK injuries in both paediatric and adult patients with head injuries and/or multiple trauma numerous factors in the failure to detect MSK injuries during the initial resuscitation and examination. Soundappan noted that more than one factor was responsible for missed injury in 30% of patients [11].

Factors divided:

a. Patient-related
b. Clinical
c. Technical
d. Radiological
e. Admission to inappropriate service

2. CASE REPORT

Our case is a 4-year-old boy brought to trauma pay by Red Crescent as a victim of a motor vehicle accident frontal impaction [10]. On arrival, ATLS primary and secondary surveys were applied and the patient was found to be free except for left thigh pain. The patient was re-examined by different ranked orthopaedic surgeons and found to have pain mainly in the distal thigh, inspection revealed no signs of local trauma, palpation resulted in tenderness over the distal thigh, the patient has a fully painful range of motion of the hip and knee joint. The patient was able to stand over his left lower limb but couldn’t proceed with walking. The distal neurovascular exam was normal.

The patient was discharged with the diagnosis of contusion. The patient visited the emergency department on two consecutive days with the same complaint of left thigh pain even at rest and inability to bear weight where he was evaluated by different orthopaedic surgeons and had the same impression of contusion [10]. At the third visit patient was brought by the family with a deformed left thigh after an unattended incident at home.

2.1 Investigations

As part of the primary survey patient’s chest and pelvis X-rays done at the trauma bay showed no abnormality. AP and lateral X-rays of the left femur showing hip and knee joints were reviewed by different ranked orthopaedics surgeons with the agreement of normal X-ray impressions (Figs. 1 & 2) [10]. The following visits for the same complaint have different X-rays with the same impression of normal X-rays (Fig. 3) [10]. Except for the one at the last visit with the clinical evidence of deformity, the patient has a diaphysial femur fracture (Fig. 4) [10].

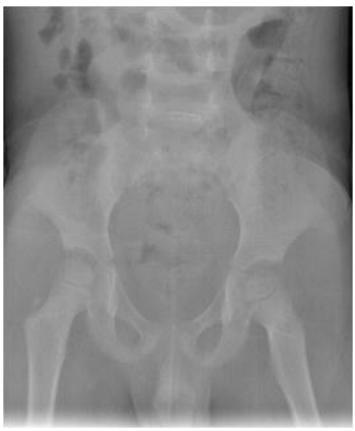

Fig. 1. Anterior, posterior pelvis x-ray showing left hip

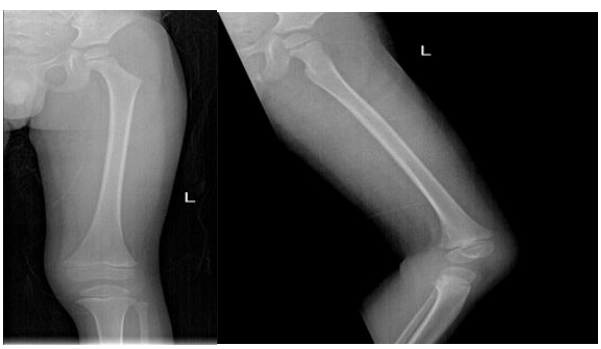

Fig. 2. Posterior, anterior and lateral x-ray of the left femur showing knee

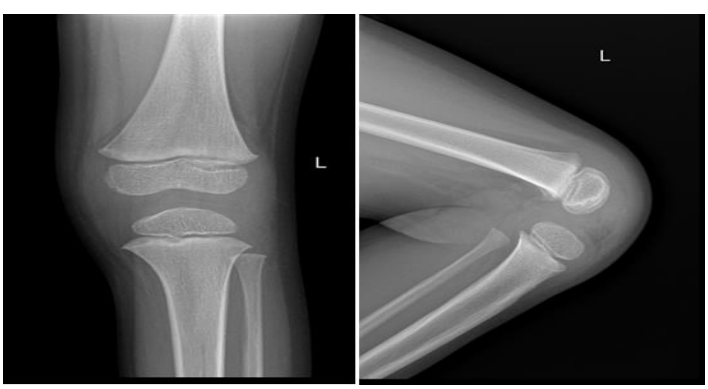

Fig. 3. Different X-rays with the same impression as normal X-rays

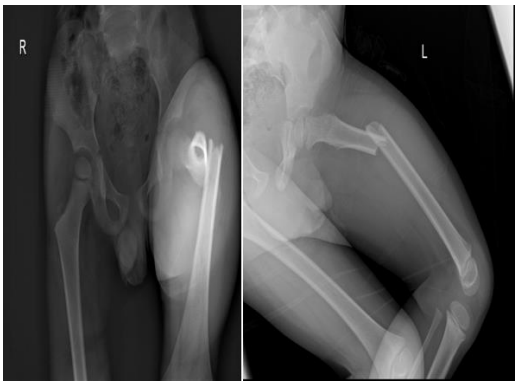

Fig. 4. X ray showing diaphysial femur fracture

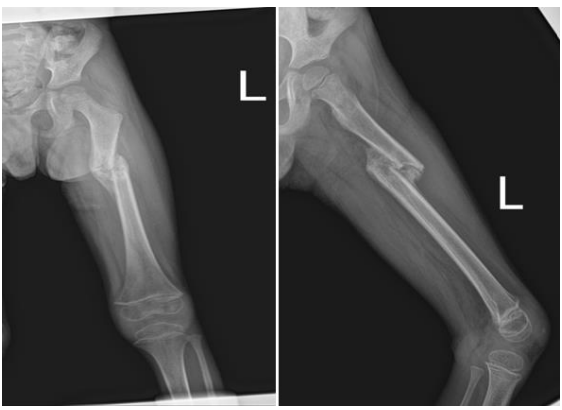

Fig. 5. X ray of healed femur fracture

2.2 Differential Diagnosis

The repetitive visits with the same complain to ER increase suspicion of injury presence [10].

Compartment syndrome and referred pain were excluded by physical examination and final diagnosis made as contusion [10].

2.3 Treatment

Patient treated primarily by reassurance, analgesia and instructions of rest and to revisit ER if pain progressed.

After evidence of fracture patient was treated with an immediate hip spica cast.

2.4 Outcome and Follow-up

Multiple OPD visits show well-aligned and healed femur fractures within the expected time frame (Fig. 5) [10].

3. DISCUSSION

Reviewing literature shows no reported similar cases. This patient has been missed diagnosis with:

a. Liable patient-related factors as he was fully conscious and reliable with repeating his complaint of thigh pain [12,10].
b. Clinical factor was also eliminated as the patient had been examined thoroughly by different doctors in different setups and times [7] .
c. Technical factors have been eliminated also as the patient has no obstacles in his clinical condition to cover his injury specifically in his limb [11].
d. Radiological factors have a deficient of only AP view in the initial Visit. However, on the second visit, proper AP and lateral views were interpreted multiple times without findings. Finally, patient hasn't been admitted under any speciality and discharged [4,10].

Learning points/take-home messages:

a. Careful listen to patients' complaints and overlook them as per described protocols
b. Suggestion of aggressive radiological investigation with repeated visit to ER
c. Prophylactic bed rest or hip brace is an option in such condition for a short period and callus might show up [10].

4. CONCLUSION

Missing fractures can happen even with most expert orthopedic surgeon. Careful history taking and complete physical examination is the key to suspect fractures or dislocations. Improper x rays or incomplete views can lead to catastrophic missed bone injuries. Remember to go with x ray principles to see 2 vies and 2 joints, joint above and joint below. The presence of distractors may lead to skipping a seen injury, these distractors include mobile calls, different social media applications, family or personal issues. Try your best to remove distractors and avoid them as much as possible. Always has clear documentation in the radiology requests by writing clear clinical indications and findings. Have direct communication with radiologist if you have a suspicious injury. If still in doubt call a senior or a colleague and discuss the case with him. Give a close follow up for those patients who may still have nonmatching clinical picture with radiology findings and see them by yourself. Allow patients to come back to the emergency department if the condition get worse. If still in doubt get an expert opinion.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available: https://medcraveonline.com/MOJOR/missed-femur-fracture-in-pediatric-trauma-patient.html#:~:text=Isolated%20paediatric%20femoral%20fracture%20is,radiological%20evaluation%20used%20for%20diagnosis

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Chapter 13
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Human Papillomavirus and Oral Lesions: A Comprehensive Review and Case Study

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and Milton Fabricio Lafebre Carrasco [b*]

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ABSTRACT

The aim of this article is to review the literature and present the case of a squamous papilloma of the soft palate. Squamous papilloma is the most common benign epithelial tumor of the oral mucosa, it is identified as an exophytic proliferation giving rise to papillary lesions with finger-like projections. Despite being a benign lesion, oral squamous papilloma is the most common benign tumor in the oral cavity. The primary cause of this tumor is the Human Papilloma Virus (HPV), which is spread through direct contact. However, recent research has shown a direct correlation between HPV infection and the development of squamous cell carcinoma in the oral cavity, making this a significant issue that needs to be addressed by various health services globally. We present the case of a 63-year-old male patient who attended the Dental Clinic of the Faculty of Dentistry of the Pontificia Universidad Javeriana Bogotá - Colombia. Through the intraoral examination, a papillary lesion on the soft palate was found. An excisional biopsy was performed and the diagnosis is confirmed by histopathological examination. The response to treatment confers a favorable prognosis and inform patients about the risk factors for developing this disease and its preventive methods.

Keywords: Papilloma; HPV; oral cavity; diagnosis.

1. INTRODUCTION

The response to treatment confers a favorable prognosis and inform patients about the risk factors for developing this disease and its preventive methods [1]. It constitutes 2.5% of the lesions of the entire oral cavity, larynx, bronchial tree,

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oesophagus, bladder, anus and genital tract [2]. Squamous papilloma shares the morphology similarity to papillary squamous cell carcinoma both clinically and histopathologically. They clinically may present a localized hyperplastic exophytic and papillary projections with finger-like proliferation or cauliflower-like morphology; there are, thus, no striking characteristic features to distinguish malignant papillary squamous cell carcinoma from benign squamous papilloma [2a]. The main etiologic factor of these lesions is the Human Papillomavirus (HPV), which comprises different types within the Papillomaviridae family, they do not contain any envelope, their diameter is 55nm, and their capsule is icosahedral (72 capsomere protein) [3]. Most HPVs that infect mucous membranes belong to the group of alpha-papillomaviruses, are considered high risk (16 and 18) represent approximately 70% of conditions of higher degree of malignant transformation while those of low risk (6 and 11) are the most common types associated with benign lesions [3,3a]. In the United States, during the years 2009 to 2010, a cross-sectional study was conducted in the civil population of patients and it was reported that the prevalence rate of oral HPV infection in men and women between 14 and 69 years of age was 6.9% and of HPV 16 was identified in 1%. A higher rate of HPV was identified in men than in women, and between 30 and 34 years and between 60 and 64 years.4 According to the site of infection, HPVs that infect the oral cavity are considered mucocutaneous [3a]. The literature associates HPV with the appearance of anogenital warts, cancer of the oropharynx, dysplasia and cervical cancer, at least 40 types of HPV can infect the skin, genitals and mucous membranes [4]. Its transmission is diverse: it can occur in the perinatal period and later in life, by sexual contact and autoinoculation, although some authors also suggest a possible transmission by saliva, oral sex has been established as the main method of transmission of HPV in mouth [5]. The lesions are limited specifically to the squamous epithelium of the mucous membranes, after the infection of the basal layer and a variable incubation time, the replication and assembly of virions occurs in conjunction with the squamous cell differentiation [3a]. When the infections are in low layers, the number of viral copies per cell is also too low to transmit the disease, in subclinical infections, viral replication of DNA and transcription are active, the observable lesions contain the active virus, these are usually exophytic, flat, papillomatous or verruciform, endophytic or less observable [6]. In the case of lesions located in the uvula or in places close to it, patients usually have difficulty swallowing food, therefore surgical excision should be performed as soon as possible [7]. This article aims to review the literature and present the case of a squamous papilloma of the soft palate.

2. CASE PRESENTATION

A 63-year-old male patient resident of the city of Bogotá, who attends the dental clinics of the Faculty of Dentistry of the Pontificia Universidad Javeriana, was referred to the oral pathology clinic [3a]. The patient with a history of Diabetes Mellitus type II diagnosed about 1 year ago controlled with Metformin 800mg twice a day, smoked 20 cigarettes a day for about 40 years. At the time of clinical examination a vegetative lesion with a pedunculated base of approximately 8mm

in diameter, whitish in color was observed at the soft palate level lateral to the base of the uvula, the patient does not know the time of evolution.

According to the clinical examination and the data obtained during the anamnesis, the following presumptive diagnosis was obtained: squamous papilloma. As a treatment, the following procedure is performed: excisional biopsy of a vegetative lesion of the soft palate. After asepsis and antisepsis, the anesthetic (lidocaine 2% with epinephrine 1: 80000) is applied at the perilesional level, the incision is made with electrocautery around the lesion and the sample is obtained. Subsequently, the sample of the lesion is placed in 10% formalin, labelled and sent for histopathological study [3a].

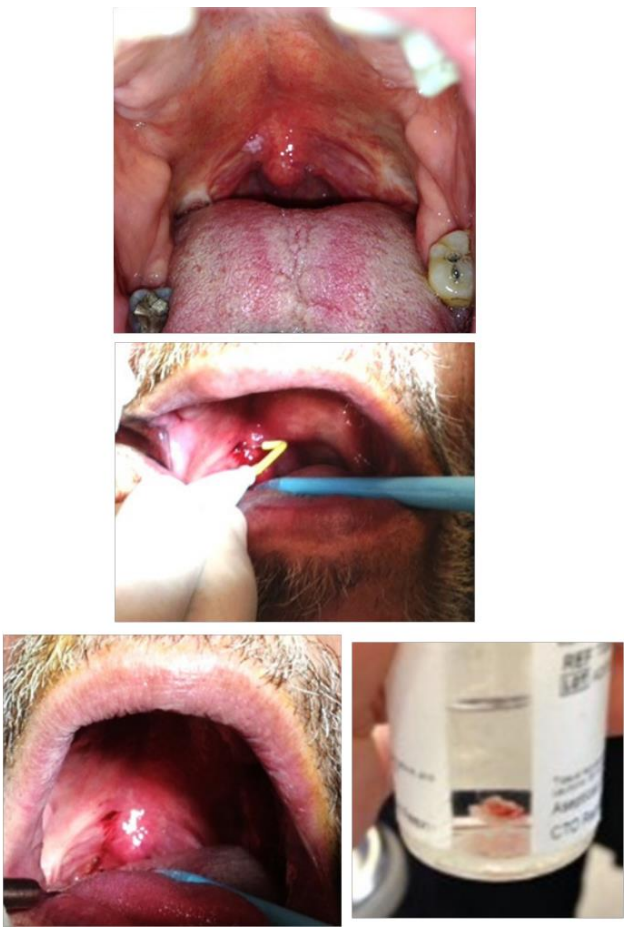

Fig. 1. Removal of the lesion, obtaining the sample

2.1 Histopathological Examination Report

Proliferation of stratified keratinized squamous epithelium, arranged in finger-like projections with fibrovascular connective tissue cores, the superficial layer of keratin is denser in lesions with a more white clinical appearance. The koilocytes are clear epithelial cells with small pyknotic nuclei [3a].

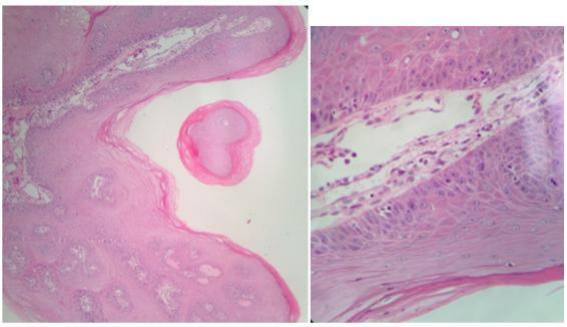

Fig. 2. Histopatological image of the lesion

Postoperative control after 8 days.

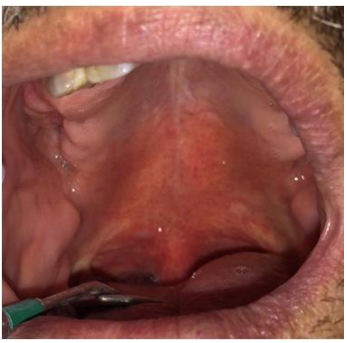

Fig. 3. Postoperative control

3. DISCUSSION

The squamous papilloma located in the oral cavity is a frequent, asymptomatic lesion, which is usually detected through a clinical examination by the dentist. Depending on the degree of keratinization, the colour of the surface of the lesion varies between red, pink or white, the most common places are the palate and the tongue, the age of presentation ranges from 20 to 50 years, with the lesions being mostly unique [3a]. Devi et al. [1] mention that in a sample of 464 squamous papillomas of the oral cavity, 34.3% of these lesions were located in the palatal complex (hard palate, soft or uvula), the majority being benign and

asymptomatic.7 Benign squamous papilloma is associated with low-risk types of HPV 6 and 11,8 affecting patients of all ages, but more often diagnosed from the second to fourth decade of life,8 which suggests that the appearance of these lesions is more frequent in people with active sexual life. However, some studies have not been able to demonstrate a correlation between the practice of oral sex and HPV infection, suggesting also that they operate non-sexual transmission mechanisms [3a]. Significant levels of HPV infection have been observed in the mouth of children aged 1 year and younger than 19 years of age, therefore studies support the vertical transmission of HPV to the oral cavity of the mother to the baby, in addition to other modes of transmission that include autoinoculation, although the lack of concordance between the HPV genotype in the oral and anal sites indicates that this is a rare modality [8]. The viral life cycle begins when the virus enters through microabrasion to the basal layer, joining the surface receptors of the basal layer (Integrin 6, Heparan Sulfate and CD69); the virions may or may not be incorporated into the nucleus of the cells, however the viral proteins E1, E2, E6, E7 are expressed at low levels; after cell division, the infected cells migrate to the suprabasal zone and begin to differentiate by activating a transcriptional cascade coordinated with the viral genome [5]. HPVs are characterized by a special tropism for squamous epithelial cells and keratinocytes. The synthesis of viral DNA and the expression of viral genes (especially for those that encode capsid proteins) are related to the level of differentiation of keratinocytes [3a]. The normal viral replication cycle is a highly regulated process, depending both on some viral proteins encoded by the viral genome and the degree of differentiation of the infected cell; the infection usually begins in the basal and para-basal cells of the squamous epithelium. Changes in keratinocytes from the basal layer to the surface of the epithelium provide a suitable micro-environment for productive cellular replication [6].

Histologically, HPV infection can be observed as an acanthotic, dyskeratotic lesion with keratinocyte multinucleation and koilocytosis. These histological features occur when the infection becomes productive, viral genes are sequentially expressed from early genes to late genes, followed by squamous epithelial differentiation, from basal and parabasal cells, where the early portions of the genome are more active and proceed to the upper layers of the epithelium (intermediate and superficial), together with the formation of the complete virion (i.e, the infected viral particle) [3a]. The classic viral cytopathic effects that may appear: koilocytosis in particular, is considered as the obvious expression of a viral cytopathic effect. The koilocytic cell shows a thicker cytoplasm at the level of the inner wall of the membrane and morphologically crashed atypical cell nucleus. Histologically, HPV infection can be observed as an acanthotic, dyskeratotic lesion with keratinocyte multinucleation and coilocytosis [6].

The most common differential diagnosis suggested was that of a condyloma. This lesion can be mistakenly considered a papilloma because the macroscopic aspect can also show a surface similar to a cauliflower. These entities can be differentiated macroscopically, microscopically and immunologically. The number of elements, the size of the lesion, the stem, the location and the colour can help distinguish them. Other similar entities include verruca vulgaris, verruciform

xanthoma, verrucous carcinoma, among others [2,3a]. The preferred treatment for squamous papilloma is surgical excision, which can be performed using different surgical techniques. One such method is cryosurgery, which involves the destruction of affected or unwanted cells and tissues using cooling elements at subzero temperatures. Cryosurgery offers several advantages, including minimal bleeding, reduced need for anaesthesia, and a faster, less traumatic postoperative recovery period [9]. The replication pattern of HPV varies depending on the type of lesions it causes. In benign lesions, the HPV genome exists in an extrachromosomal episomal (plasmid) form, while in malignant lesions, viral DNA becomes integrated into the host genome [10]. Regarding the association of HPV with oral cavity cancer development, it is observed that the integration of the viral genome into the host cell is a rare occurrence. When integration does happen, it typically affects the host chromosomes at fragile sites. Following this transformation into carcinogens, the neoplastic process advances rapidly [3a]. The integration of HPV in the host genome induces the increase of the expression of E6 and E7 proteins, since the integration results, produce the interruption of the E2 gene of HPV, which is a negative regulator of the transcription of proteins E6 and E7. High-risk HPV E6 and E7 oncoproteins can independently induce genomic instability in normal human cells [11]. As expressed above, the incidence rates of the base of tongue cancer and tonsil have increased gradually, according to data from the National Cancer Institute, Epidemiology in the United States, from 1973 to 2001 it was documented that base of tongue cancer and palatine tonsils increased between 2 to 4% annually, these two locations being the most frequent [12,3a].

HPV seems to have tropism by Waldeyer's lymphoepithelial ring, in lingual and palatine tonsils, in these areas there are deep intussusceptions (tonsillar crypts), in which, the immature cells are more easily exposed to HPV, so the lesions are more frequent in these areas [3a]. More recent studies obtained data in North America on the incidence of cancer of the oral cavity and oropharynx, with lower percentages of HPV-16 in carcinomas of the oral cavity (0-4%), while higher percentages were observed in cancers of the oropharynx (63-82).%), these findings suggest that HPV-16 is more likely to be detected in the oropharyngeal territory than in the oral cavity [13].

Current studies indicate a rise in cases of oropharyngeal cancer among middle-aged men in recent years, with a significant proportion linked to HPV types 16 and 18 (approximately 70%). Reggrettably, a considerable number of these cases are diagnosed in elderly individuals (aged 60 and above), resulting in lower treatment success rates and heightened morbidity and mortality in this demographic [14].

Despite these challenges, the prognosis improves significantly when treatments are administered for HPV-positive cancers compared to those not linked to viral infection. The detection of tumour viral DNA (ct)DNA proves valuable in determining the prognosis, progression or recurrence of patients with HPV-associated oropharyngeal cancer [15].

Within the preventive plan, at present two vaccines protect against HPV 16 and 18, which cause 70% of cases of carcinoma of the uterine cervix. Vaccines may confer some cross-protection against other less common types of HPV that are also the cause of this cancer [3a]. One of the vaccines also protects against types 6 and 11, which cause anogenital warts.

The bivalent vaccine called (Cervarix) offers protection against HPV types 16 and 18, the accepted indications for the bivalent vaccine are only cervical cancer and its precursors.

The quadrivalent vaccine (Gardasil) includes protection against HPV 6 and 11 because genital warts are benign mucosal lesions of the mucosa caused by the human papillomavirus (HPV), most commonly types 6 and 11 [3a]. These lesions are part of one of the most common viral sexually transmitted infections and are very common in people with more than 10 sexual partners throughout their life or in sexual partners, where at least one had genital warts, the use is limited to cancers of the vulva, the vagina and the anus and their precursors [16]. Regarding the oral cavity, until 2015, only 9 cases have been reported in which the quadrivalent vaccine was applied in patients with oral lesions associated with HPV, with encouraging results in short-term patients, however in patients with immune alterations, the effectiveness of the vaccine on present lesions decreases considerably [17]. Despite this, in the preventive field, in the few studies that are being carried out to date, a reduction in the prevalence of oral HPV 16 - 18 lesions is observed among vaccinated women, however, are still questioned because the indication of the vaccine is based on the epidemiology of HPV in the cervix, so it is necessary to carry out studies that provide definitive evidence for the use of the vaccine as a method preventive in the development of oral cancer [15,3a].

In recent years, multiple studies have shown that HPV vaccination reduces the prevalence of HPV in the oral cavity, offering a significant preventive benefit to the population, particularly men [18], who face a higher risk of contracting the virus, possibly due to engaging in risky sexual behaviours associated with promiscuity.

Numerous authors have pointed out that the administration of the quadrivalent vaccine (Gardasil) triggers the production of anti-HPV 16 and 18 antibodies. These antibodies reach their peak levels in the oral cavity approximately one month after the third dose (seventh month after vaccination initiation). However, antibody levels tend to decline between 18 and 30 months post-vaccination, while remaining stable in blood serum.

While a study conducted by Parker et al. supports these findings, they recommend further research to gather conclusive data on the efficacy of vaccination in preventing HPV-associated oropharyngeal cancer [19].

4. CONCLUSION

Squamous papilloma is a common benign lesion among patients attending dental practice, however, according to recent studies, HPV is closely associated with the development of oral squamous cell carcinoma, therefore the dentist is under an obligation to examine the oral cavity of patients looking for lesions that may indicate neoplastic process caused by HPV, since in the early stages, the response to treatment confers a favourable prognosis and inform patients about the risk factors for developing this disease and its preventive methods [3a].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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