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Awareness about Modified Adenosine among Allied Health Science Students

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Introduction: N6-methyl-adenosine (m6A) is the most abundant modification in mammalian mRNA and long non-coding RNA. First discovered in the 1970s, m6A modification has been proposed to function in mRNA splicing, export, stability, and immune tolerance. Interest and excitement in m6A modification has recently been revived based on the discovery of a mammalian enzyme that removes m6A and the application of deep sequencing to localise modification sites. The m6A demethylase fat mass and obesity associated protein (FTO) controls cellular energy homeostasis and is the first enzyme discovered that reverses an RNA modification. m6A Sequencing demonstrates cell-type- and cell-state-dependent m6A patterns, indicating that m6A modifications are highly regulated. Aim: This survey was conducted for assessing the awareness about modified adenosine among Allied Health Science students. Materials and method: A cross-section research was conducted with a self-administered questionnaire containing ten questions distributed amongst 100 allied Health Science students. The questionnaire assessed the modified adenosine among Allied Health Science Students. The responses were recorded and analysed. Results: 65.3% of the respondents were aware of modified adenosine . 58.4% were aware about it's clinical manifestations . 65.3% were aware of the causes of Reversal of modified Adenosine . 61.4% were aware that m6A can alter local RNA structures . 59% were aware of m6A "readers" . Conclusion: There is moderate awareness amongst Allied Health Science students about Modified Adenosine. Enhanced awareness initiatives and educational programmes together with the increased importance of curriculum and improvements that further promote knowledge and awareness of Modified Adenosine among Allied Health Science students.

Keywords: Awareness, students, modifiedadenosine, allied health science.

1. Introduction

N6-methyl-adenosine (m6A) is the most abundant modification in mammalian mRNA and long non-coding RNA. First discovered in the 1970s, m6A modification has been proposed to function in mRNA splicing, export, stability, and immune tolerance. Interest and excitement in m6A modification has recently been revived based on the discovery of a mammalian enzyme that removes m6A and the application of deep sequencing to localise modification sites. The m6A demethylase fat mass and obesity associated protein (FTO) controls cellular energy homeostasis and is the first enzyme discovered that reverses an RNA modification. m6A Sequencing demonstrates cell-type- and cell-state-dependent m6A patterns, indicating that m6A modifications are highly regulated.(1)

Ribonucleoside analogues bearing terminal alkynes are useful for RNA modification applications. The alkyne serves as a substrate for copper-catalysedazide/alkyne cycloaddition (CuAAC or "click") reactions, allowing for further manipulation of the RNA structure and its properties. Terminal alkynes can be incorporated into RNA enzymatically or using modified phosphoramidites. Using the latter approach, researchers were able to tune the properties of an siRNA with 7-ethynyl-8-aza-7-deazaadenosine (7-EAA) as well as 1,2,3-triazoles from CuAAC reactions of a 7-EAA-containing guide strand.(2)

New messenger RNA (mRNA) transcripts require additional processing and modifications before translation and protein synthesis. RNA modifications regulate most steps of gene expression, from indirectly controlling DNA transcription through transcription factors, to directly affecting mRNA translation . Many RNA modifications have been uncovered thanks to next generation sequencing technologies. Some RNA modifications are difficult to study since there may be an inability to distinguish between certain nucleotides.(3)

Efficient in vitro transcription methods using T3, T7, and SP6 RNA polymerases have been widely used to produce RNA for a variety of applications. Commonly used in vitro transcription systems all use guanosine and its nucleotides for transcription initiation.Adenosine receptors are involved in many physiological processes and pathological conditions and are therefore attractive therapeutic targets. To identify new types of effective ligands for these receptors, a library of adenosine derivatives bearing a boron cluster or phenyl group in the same position was designed. The ligands were screened in silico to determine their calculated affinities for the A2A and A3 adenosine receptors.(4)

N6-methyladenosine (m6A) modification in mRNA is extremely widespread, and functionally modulates the eukaryotic transcriptome to influence mRNA splicing, export, localization, translation, and stability. Methylated adenines are present in a large subset of mRNAs and long noncoding RNAs (lncRNAs). Methylation is reversible, and this is accomplished by the orchestrated action of highly conserved methyltransferase (m6A writer) and demethylase (m6A eraser) enzymes to shape the cellular 'epitranscriptome'. The engraved 'methyl code' is subsequently decoded and executed by a group of m6A reader/effector components, which, in turn, govern the fate of the modified transcripts, thereby dictating their potential for translation.(5)This survey was conducted for assessing the awareness about modified adenosine among Allied Health Science students.

2. Materials and methods:

This cross - sectional research was conducted with a self - administered questionnaire containing ten questions distributed amongst 100 Allied Health science students.The Students were randomly selected across various disciplines of Allied Health Sciences. The Study setting was designated in the university campus.The survey instrument was a questionnaire pre-tested and evaluated for validity and reliability concerns.

The questionnaire included ten questions eliciting the demographic data through open-ended responses and multiple choice questions for the other responses. The study was approved by the Institutional Ethical Committee and informed consent was obtained from the participants. The questionnaire was posted in an online platform and the identity of the respondents were kept confidential.

The questionnaire assessed the awareness about modified adenosine, clinical manifestations of modified adenosine, causes of complications of modified adenosine, and prevention methods .The responses were recorded and analysed.

The salient questions in the study are

1. Are you aware of modified Adenosine?
2. Do you have any knowledge about it's clinical manifestations?
3. Are you aware of the causes of Reversal of modified Adenosine?
4. Are you aware that m6A can alter local RNA structures?
5. Are you aware of m6A "readers"?

3. Result:

65.3% of the respondents were aware of modified adenosine (Fig 1). 58.4% were aware about it's clinical manifestations (Fig 2). 65.3% were aware of the causes of Reversal of modified Adenosine (Fig 3). 61.4% were aware that m6A can alter local RNA structures (Fig 4). 59% were aware of m6A "readers" (Fig 5).

Fig :1 Awareness about modified Adenosine

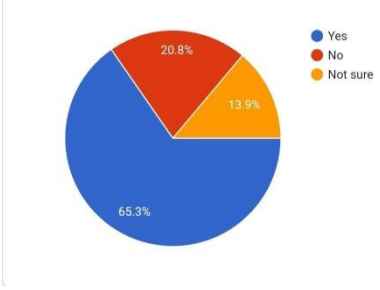


Fig :2 Awareness about the clinical manifestations of modified adenosine

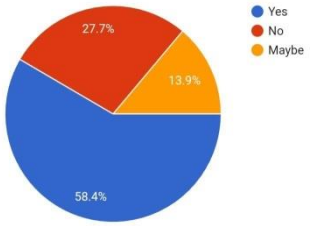


Fig :3 Awareness about the causes of Reversal of modified Adenosine

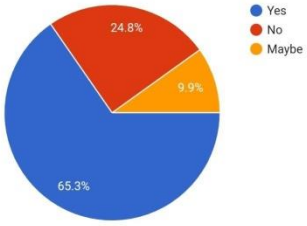


Fig :4 Awareness about m6A that can alter local RNA structures

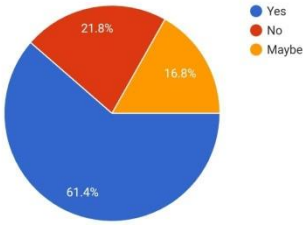
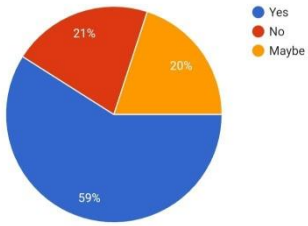


Fig :5 Awareness about m6A "readers"



4. Discussion:

N6-methyladenosine (m6A) modification in mRNA is extremely widespread, and functionally

modulates the eukaryotic transcriptome to influence mRNA splicing, export, localization, translation, and stability. Methylated adenines are present in a large subset of mRNAs and long noncoding RNAs (lncRNAs). Methylation is reversible, and this is accomplished by the orchestrated action of highly conserved methyltransferase (m6A writer) and demethylase (m6A eraser) enzymes to shape the cellular 'epitranscriptome'. (6) 65.3% of the respondents in this study were aware of modified adenosine.

Adenosine deaminase 2 deficiency (DADA2) is a complex systemic autoinflammatory disorder in which vasculopathy/vasculitis, dysregulated immune function, and/or hematologic abnormalities may predominate. Inflammatory features include intermittent fevers, rash (often livedoracemosa/reticularis), and musculoskeletal involvement (myalgia/arthralgia, arthritis, myositis). Vasculitis, which usually begins before the age of ten years, may manifest as early-onset ischemic (lacunar) and/or hemorrhagic strokes, or as cutaneous or systemic polyarteritisnodosa. Hypertension and hepatosplenomegaly are often found. More severe involvement may lead to progressive central neurologic deficits (dysarthria, ataxia, cranial nerve palsies, cognitive impairment) or to ischemic injury to the kidney, intestine, and/or digits. (7)

Reversal of m6A is performed by at least two demethylase "erasers" that have been present. Fat mass and obesity-associated protein (FTO) is associated with human body weight regulation while ALKBH5 is associated with fertility in mice The reversibility of the m6A modification suggests a dynamic role for the modification in controlling the fate of modified RNAs. m6A modifications are present to modulate eukaryotic translation efficiency , cap-independent translation , mRNA stability , RNA splicing and miRNA biogenesis. (8)

m6A can alter local RNA structures, for example, by destabilising RNA helices. Thus, m6A can affect RNA stability or possibly RNA localization. Additionally, heterogeneous ribonucleoproteins (hnRNP) have recently been shown to interact with m6A-modified RNA. The presence of m6A modifies local RNA structure, which promotes the nuclear protein hnRNP C to bind to this region and to modulate pre-mRNA processing. The nuclear localization of these RNA-binding proteins suggests a role in the life cycle of viruses that replicate their genomes in the nucleus, such as influenza virus. (9)

Proteins that selectively bind m6A sites are defined as m6A "readers", which exert regulation by influencing the recognition of methylated RNA. It has been established that the YTH N6-methyladenosine RNA-binding protein family comprising YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2 is the major protein family among all "readers".(10-12) There is moderate awareness among allied health science students about m6A "readers" as assessed in this study.

Modified adenosine, which includes various chemical alterations to the adenosine base in DNA or RNA, can significantly impact gene regulation and cellular functions, potentially playing a key role in the development of oral diseases. These modifications, such as N6-methyladenosine (m6A) in RNA or N6-carbamoyl methyladenine in DNA, act as epigenetic marks that regulate gene expression, influencing cellular processes like proliferation, differentiation, and apoptosis. In oral cancers, such as squamous cell carcinoma, altered adenosine modifications could disrupt the expression of oncogenes or tumor suppressor genes, promoting tumor growth and metastasis. Similarly, in inflammatory diseases like periodontitis,

these modifications may affect the regulation of immune responses, potentially leading to chronic inflammation or impaired resolution of inflammation. Additionally, adenosine modifications play a role in cellular stress responses, which are crucial in protecting oral tissues from damage; however, if dysregulated, they could increase susceptibility to disease. The oral microbiome might also interact with modified adenosine, influencing host-microbe dynamics and potentially contributing to disease development. Understanding the role of modified adenosine in oral diseases could open new avenues for therapeutic interventions, allowing for the correction of aberrant gene expression patterns and offering novel approaches to treating conditions like oral cancer and chronic inflammatory diseases.(13-17)

5. Conclusion

There is moderate awareness amongst Allied Health Science students about Modified Adenosine. Enhanced awareness initiatives and educational programmes together with the increased importance of curriculum and improvements that further promote knowledge and awareness of Modified Adenosine among Allied Health Science students.

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