

# Assessment of Glycoconjugates Levels as Liability in Oral Precancerous and Oral Cancer Patients

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## Abstract:-

### ➤ Background:

Because glycoproteins are crucial for maintaining the integrity of cells, dysglycoproteinemia has long been associated with cancer patients. The objective is to assess and correlate the serum glycoprotein levels in patients with oral cancer and possibly malignant illnesses. **RESOURCES AND METHODS:** There are 75 participants in this study, divided into three groups. **Group 1:** mouth cancer comprises 25 participants who have received a diagnosis of mouth cancer confirmed by histopathology. **Group 2:** Oral precancer comprises 25 participants who have been diagnosed with oral possibly malignant based on a histopathological confirmation. **Group 3:** A controlled group of twenty-five healthy volunteers, matched for age and sex.

### ➤ Outcomes:

The individuals in the OPM and OC group had significantly greater mean serum levels of protein bound hexose, total sialic acid, and fucose as compared to control.

**Keywords:-** *Dysglycoproteinmia, Oral Potentially Malignant, Serum PBH, Serum Total Sialic Acid, Serum Fucose.*

Greek word "Proteios," which meaning "primary or holding first place," is the source of the term "protein." These substances, as their name suggests, are the most significant components of cells. They are universally found in the cytoplasm and cell membrane of all cells. In addition, proteins can be found in viruses, hormones, enzymes, antibiotics, and other substances. There are three categories for proteins: derived, conjugated, and simple. These are the end results of partially digested or denaturised proteins. Example: Met-proteins, Coagulated Proteins, and Proteins. Protein-carbohydrate complexes known as glycoproteins are made up of oligosaccharides or polysaccharides that are covalently linked to particular amino acids of proteins. These substances include blood group substances, enzymes, hormones, cell membranes, mucous secretions, and antibiotics. Amino sugars such as glucose, galactosamine, or sialic acid, as well as hexose (galactose, mannose), or fucose, are found in the carbohydrate component. Malignant cells produce more glycoconjugates through enhanced turnover, secretion, and seeding, which releases them into circulation. Elevated levels of glycoconjugates, such as total sialic acid and lipid-bound hexose, have been found in the serum of patients suffering from various types of cancer, including malignant melanoma, ovarian cancer, Hodgkin's lymphoma, and oral cancers. These findings suggest that these glycoconjugates may be useful in diagnosing or tracking treatment.

## I. INTRODUCTION

One of the most serious health issues affecting people today is cancer. Hippocrates, a Greek physician, is credited with coining the phrase cancer. Worldwide, oral cancer is a major health concern. It has been noted that the Indian subcontinents are known for their addictive behaviours, such as chewing tobacco and betel nut, due to cultural, ethnic, and geographic factors. According to WHO estimates, it is the sixth most prevalent cancer globally, with two thirds of occurrences occurring in developing nations. The South Asian region has the highest incidence rates of mouth cancer globally. India has often been mentioned as the nation with the highest global prevalence. More than 100,000 instances are reported annually in India alone.

## II. MATERIALS AND METHODS

### A. Study Subjects:

There are 75 volunteers in this study, divided into three groups. **Group 1:** mouth cancer comprises 25 participants who have received a diagnosis of mouth cancer confirmed by histopathology. **Group 2:** Oral precancer is made up of 25 individuals who have had their diagnosis of oral precancer confirmed by histopathology. **Group 3:** A controlled group of twenty-five healthy volunteers, matched for age and sex.

### B. Sampling:

The research was carried out in the Department of General Biochemistry, Annamalai University, and the Department of Oral and Maxillofacial Pathology, Rajah Muthiah Dental College. All patients' personal and demographic information was entered into pre-designed Performa with their previous informed consent. Following informed consent, biopsy samples from patients suspected of having oral cancer and precancer were taken, and their samples were examined histopathologically. Blood samples from patients with precancer lesions and/or conditions, mouth cancer patients (oral squamous cell carcinoma), and healthy individuals are included in the sample.

### C. Sample Collection:

Using a venipuncture in the cubital fossa, two milliliters of blood were drawn from each participant and placed into a sterile, disposable glass vacutainer tube. To prevent external damage, the samples were stored in ice packs and allowed to coagulate in an incubator at 37 degrees Celsius for approximately one hour. To separate the serum, the blood was centrifuged for five minutes at 3000 revolutions per minute (rpm). It was then kept in an ultra-low temperature freezer at -81 degrees Celsius until analysis.

#### ➤ Materials Utilized in Protein Estimation.

- Test tubes kept sterile.
- Syringe with 2 millilitres.
- A 2-milliliter vial tube.
- An oven with hot air.
- A refrigerator.
- A hot bath.
- The Spectrophotometer. UV-VIS.
- A measuring tubes

## III. METHOD

### A. Glycoprotein Extraction

The amount of protein-bound hexose was estimated using the precipitate that was left behind after the serum was treated with 95% ethanol. After lipid extraction, the dry defatted tissues were added, and for an hour, they were hydrolysed at 80°C with 0.1N H<sub>2</sub>SO<sub>4</sub>. The sample was used for sialic acid measurement after it had cooled. Fucose was measured after adding 0.1N sodium hydroxide to the residual solution and letting it sit in an ice bath for an hour.

### B. Protein Bound Hexose Estimation

The "Niebes" method was used to measure the amount of protein bound hexose in serum, erythrocyte membrane preparation, and defatted tissues. Orcinol and sulfuric acid reagent were added to the glycoprotein extract, and it was heated to 80°C for 15 minutes. At 540 nm, the colour developed was measured chemicals.

- 5% P
- 1.5% Phenol.
- Solubric acid concentrated.
- Galactose–mannose standard: galactose and mannose at 0.2 and 0.1 mg/ml, respectively.

#### ➤ Process

- 2.5 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added to 0.5 ml of sample and 0.5 ml of 5% phenol. The same procedure was applied to standards in the concentration range of 40–200 µg and 0.5 ml of 0.1 N NaOH for the blank. After 20 minutes of heating the tubes in a boiling water bath, the absorbance at 490nm was recorded. For serum, the amount of protein-bound hexose is reported as milligrams per decilitre.

### C. Fucose Estimation

The "Dische" and "Shettles" method was used to estimate the glucose. Cysteine hydrochloride is added to the sample after it has been heated with H<sub>2</sub>SO<sub>4</sub> for ten minutes in this procedure. At 430 nm, the colour developer's absorbance was measured.

#### ➤ Agents

- 95% of it is ethanol.
- 0.1 N sodium hydroxide.
- Sulfuric acid-water mixture: one volume of water and six volumes of concentrated sulfuric acid.
- The cysteine hydrochloride reagent, which is 3 g of the compound in 100 ml of water.
- Stock standard: 100 millilitres of distilled water were mixed with 50 milligrams of fucose.
- Operational norm to achieve a 50 µg/ml concentration, 10 ml of stock standard was diluted to 100 ml using distilled water.
- Process: A combination of sulfuric acid and water (4.5 ml) was introduced to a 0.5 ml sample. After three minutes in a bath of boiling water, the tubes were cooled. The reagent cysteine hydrochloride was introduced in 0.1 ml. The same procedure was applied to 0.5 ml of 0.1 N NaOH for the blank and standards with concentrations between 5 and 25g. At 430 nm, absorbance was measured after 75 minutes in the dark. For serum, the fucose content concentration is given as milligrams per decilitre.

### D. Estimation of Total Sialic Acid

Warren's (1959) method was used to estimate the total sialic acid in the serum.

#### ➤ Agents

- Periodic acid: 0.025M in sulfuric acid (0.1N).
- Meta-arsenate of sodium: 4% in 0.5N HCl.
- Thiobarbituric acid (TBA): 10 millilitres of hot distilled water dissolved 144 milligrams of TBA.
- Acidified butanol: n-butanol with 5% HCl.

- Standard Sialic acid (0.2 mg/ml): 100 millilitres of pure water were used to dissolve 179 milligrams of Orosomucoid.
- Fundamental: A yellow-coloured substance is produced when periodate is used to oxidize the sialic acid sample. Heating this with Thiobarbituric acid results in the formation of a complex with a pink colour. The absorbance of this coloured complex is measured at 540 nm after it is extracted using acidified butanol. Using a calibration curve, the intensity is compared to a known concentration of standards to determine the quantity of sialic acid present in the sample. Bathe in boiling water for precisely six minutes. After cooling, 5ml of butanol that had been acidified was added. At 540 nm, the extract's absorbance in the organic layer was measured in comparison to the reagent blank. For serum, the total sialic acid content is reported as milligrams per decilitre.

**IV. RESULTS**

In the present study, out of 75 subjects (25 precancer, 25 cancer and 25 controls), 41(54.66%) were males and 34(45.33%) were females with age range of 19-80 years with mean age of 56.32 ±12.28 (mean ± SD).

The oral cancer group comprised of 13(52%) males and 12(48%) females out of 25 subjects, with age ranges of 35-86 years with mean age 56.32 ± 12.28 years.

The oral precancer group comprised of 15 (60%) males and 10 (40%) females out of 25 subjects with age ranges of 17-71 years with mean age 43.16 ±12.49 years.

The control group comprised of 13(52%) males and 12(48%) females out of 25 subjects, with age ranges of 32-80 years with mean age 56.32 ± 12.28 years.

All the subjects studied were between the ages of 17-86 years within the mean age of 56 ±12 (mean ± SD).

The Glycoproteins in the form of Protein bound hexose (PBH), Total sialic acid (TSA) and Fucose were estimated in each and every subject of this study. The mean glycoproteins values of oral precancer, cancer and control groups are shown in Table 1. Significant increase levels of mean serum PBH, TSA and Fucose is found in the subjects of OPC and OC group as compared with control group; however, the mean glycoproteins levels were also significant.

In the study of proteins, out of 75 subjects (25 precancer, 25 cancer and 25 controls), 41(54.66%) were males and 34(45.33%) were females with age range of 19-80 years with mean age of 56.32 ± 12.28 (mean ± SD).

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All the subjects studied were between the ages of 17-86 years within the mean age of 56 ±12 (mean ± SD).

Table 1: Over all Comparison of Mean, SD of Serum Fucose, Serum Protein Bound Hexose, Total Sialic Acid Levels and Overall, in Normal by ANOVA Test

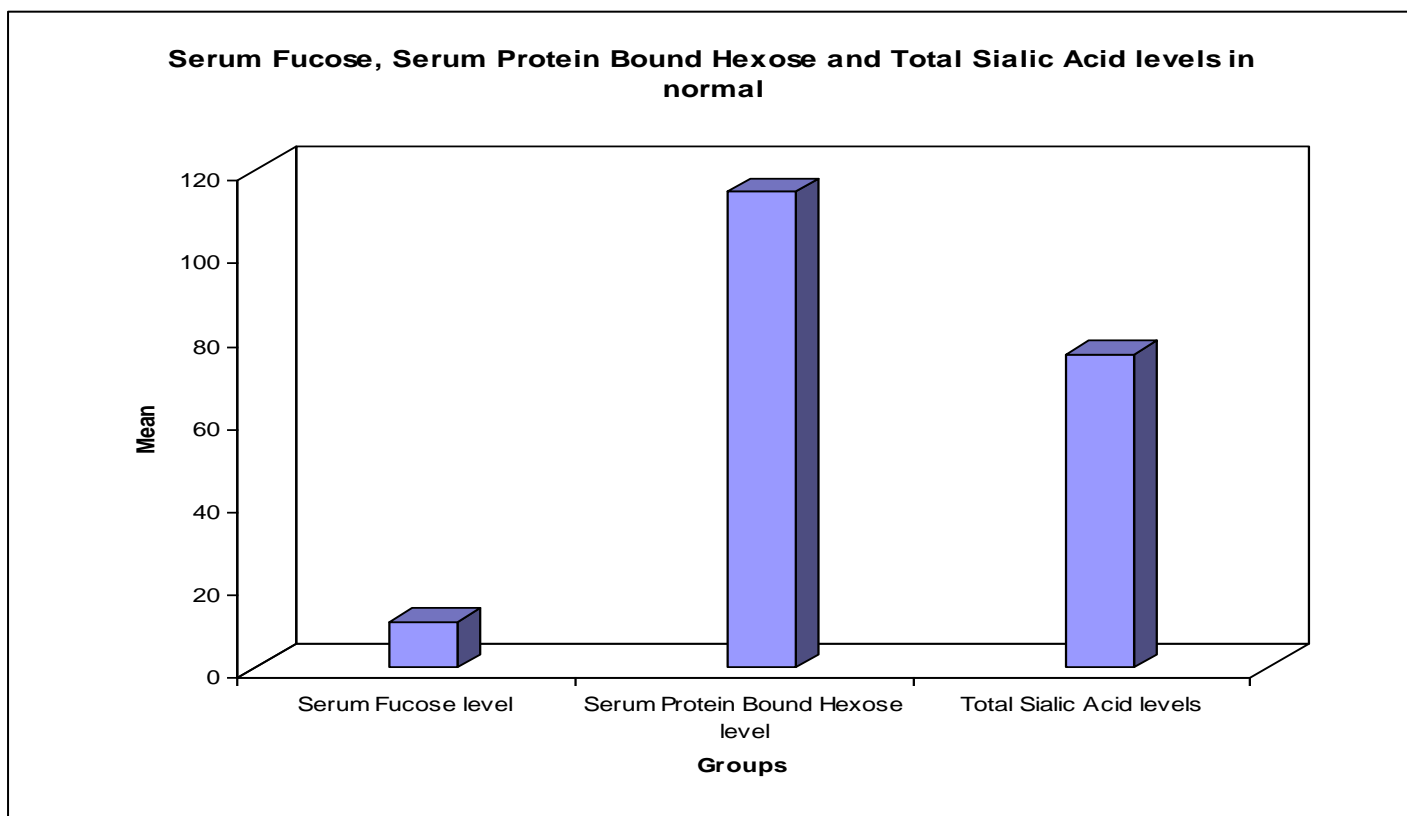
Groups	N	Mean	SD	F-value	P value
Serum Fucose level	25	10.92	1.65	1730.54	0.001, S
Serum Protein Bound Hexose level	25	114.84	7.78		
Total Sialic Acid levels	25	75.64	7.48		
Overall	75	67.13	43.58		

P < 0.01, Significant in all Groups  
P-Value, Probability Value

Table 2: Comparison of Mean, SD of Serum Fucose-Serum Protein Bound Hexose, Serum Fucose-Total Sialic Acid Levels, Serum Protein Bound-Total Sialic Acid and Overall, in Normal by Using t Test

Groups	N	Mean	SD	t-value	P value
Serum Fucose level	25	10.92	1.65	65.27	0.001, S
Serum Protein Bound Hexose level	25	114.84	7.78		
Serum Fucose level	25	10.92	1.65	42.23	0.001,S
Total Sialic Acid levels	25	75.64	7.48		
Serum Protein Bound Hexose level	25	114.84	7.78	18.15	0.001,S
Total Sialic Acid levels	25	75.64	7.48		

P-Value, Probability Value



Graph 1: Comparison of Mean, SD of Serum Total Sialic Acid, Fucose and Protein Bound Hexose Level in Controls is Done by using ANOVA test. All the Groups Show Significant Values ( $p < 0.001$ ) and ( $f < 1730.54$ ). The Values Show Significantly Increasing in Serum Protein Bound Hexose and Sialic Acid in Normal.

Comparison of Mean, SD in between of serum Fucose-serum Protein bound, serum Fucose-Total sialic acid and serum Protein Bound-Total sialic acid levels in normal by

*student t-test*. All the groups show significant ( $p < 0.001$ ) and t-Value. The values show significantly increasing in normal.

Table 3: Overall Comparison of Mean, SD of Serum Fucose, Serum Protein Bound Hexose and Total Sialic Acid Levels in Pre Cancer by ANOVA Test

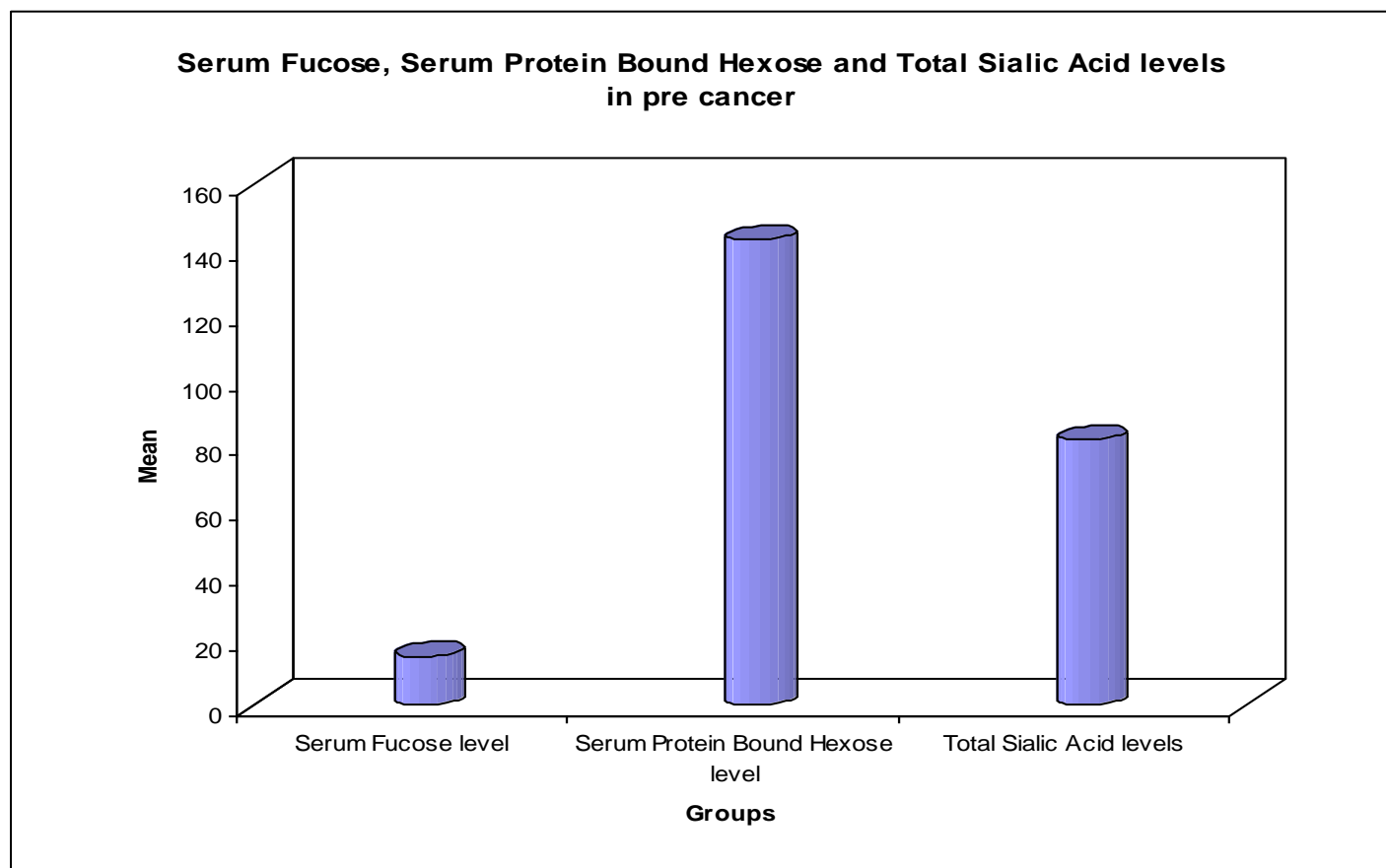
Groups	N	Mean	SD	F-value	P value
Serum Fucose level	25	14.96	1.85	1682.54	0.001,S
Serum Protein Bound Hexose level	25	142.88	10.47		
Total Sialic Acid levels	25	81.44	8.33		
Overall	75	79.76	53.15		

$P < 0.001$ , Significant in all Groups  
P-Value, Probability Value

Table 4: Overall Comparison of Mean, SD in between Serum Fucose-Serum Protein Bound Hexose, Serum Fucose-Total Sialic Acid and Serum Protein Bound-Total Sialic Acid Levels in Precancer by t Test

Groups	N	Mean	SD	t-value	P value
Serum Fucose level	25	14.96	1.85	60.16	0.001, S
Serum Protein Bound Hexose level	25	142.88	10.47		
Serum Fucose level	25	14.96	1.85	38.95	0.001, S
Total Sialic Acid levels	25	81.44	8.33		
Serum Protein Bound Hexose level	25	142.88	10.47	22.96	0.001, S
Total Sialic Acid levels	25	81.44	8.33		

P-Value, Probability Value



Graph 2: Comparison of Mean, SD of Serum Fucose, Serum Protein Bound Hexose, Total Sialic Acid and Overall Levels in Precancer Patients is done by using ANOVA Test. All the Groups Show Significant Values ( $p < 0.001$ ) and ( $f < 1682.54$ ). The Values Show Significantly Increased in Precancer Compare to Controls.

Comparison of Mean, SD of serum Fucose-serum Protein bound Hexose serum Fucose-Total sialic acid, serum protein bound Hexose-Total sialic acid levels in precancer by

student t-test. All the groups show significant ( $p < 0.001$ ) and t-Value. The values show significantly increased in precancer compared to controls.

Table 5: Overall Comparison of Mean, SD for Serum Fucose, Serum Protein Bound Hexose and Total Sialic Acid Levels in Cancer Patients by ANOVA Test

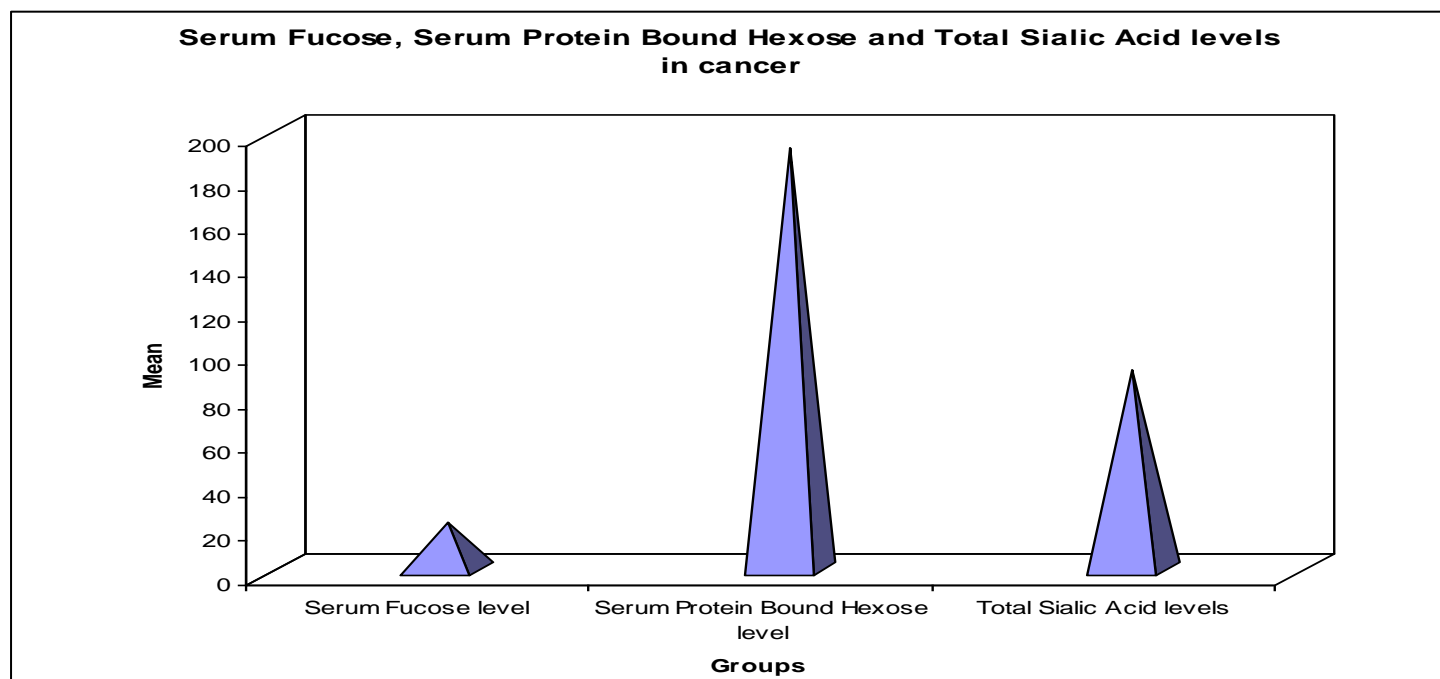
Groups	N	Mean	SD	F-value	P value
Serum Fucose level	25	20.93	1.89	1305.57	0.001,S
Serum Protein Bound Hexose level	25	192.64	19.23		
Total Sialic Acid levels	25	91.20	7.39		
Overall	75	101.59	71.93		

$P < 0.001$ , Significant in all Groups  
P-Value, Probability Value

Table 6: Overall Comparison of Mean, SD in between Serum Fucose-Serum Protein Bound Hexose, Serum Fucose-Total Sialic Acid and Serum Protein Bound-Total Sialic Acid Levels in cancer by using t Test

Groups	N	Mean	SD	t-value	P value
Serum Fucose level	25	20.93	1.89	44.43	0.001, S
Serum Protein Bound Hexose level	25	192.64	19.23		
Serum Fucose level	25	20.93	1.89	46.03	0.001, S
Total Sialic Acid levels	25	91.20	7.39		
Serum Protein Bound Hexose level	25	192.64	19.23	24.62	0.001,S
Total Sialic Acid levels	25	91.20	7.39		

P-Value, Probability Value



Graph 3: Comparison of Mean, SD of Serum Fucose, Serum Protein Bound Hexose, Total Sialic Acid and Overall Levels in Cancer Patients is done by using ANOVA test. All the Groups Show Significant Values ( $p < 0.001$ ) and ( $f < 1305.57$ ). The Values Show Significantly Increased, in Cancer Compare to Controls.

Comparison of Mean, SD of serum Fucose-serum Protein bound Hexose serum Fucose-Total sialic acid, serum protein bound Hexose-Total sialic acid levels in cancer by student t-test. All the groups show significant ( $p < 0.001$ ) and t-Value. The values show significantly increased in cancer groups.

## V. DISCUSSION

It has long been hypothesized that changes occur on the cell surface when a normal cell turns cancerous. Numerous alterations to the cell membrane, including as altered glycoproteins, modifications to surface enzymes, and further phenotypic abnormalities, have been linked to the malignant transformation of a cell. Determining the molecular alterations in cell-surface glycoproteins that occur during malignant transformation has received particular interest (Warren L., et al). When comparing oral malignant and oral precancerous patients with controls, our study found a substantial rise in glycoconjugates such as total sialic acid, serum fucose level, and protein bound hexose level ( $p < 0.001$ ). This is in line with research by Manoharan et al. (2003) and Neeraj T et al. (2009), wherein they discovered that OSCC patients had statistically higher levels of glycopeptides such PBH, TSA, and SFL. According to this findings and other research involving malignant tumours, glycoconjugate levels are higher in malignant tissue relative to normal tissues. This has given us justification to consider serum or plasma glycoconjugate levels as potential indicators for early cancer diagnosis.

Out of the three glycoconjugates examined, mouth cancer and oral precancerous patients' sera had somewhat higher PBH levels than those of normal controls ( $p < 0.001$ ). This nearly agrees with the research conducted by Manoharan

et al. (2003) ( $P < 0.001$ ) study. It was discovered that the presence and severity of malignant disease correlated with the higher glycoconjugate concentrations (Xing RD et al., 1994). Glycoconjugates are released from the cell membrane, which has been demonstrated to have higher turnover in oral cancer patients, accounting for the elevated levels of glycoconjugates in the sera of these patients (Rao VR et al., 1998). The release of glycoprotein from the erythrocyte membrane or the tumour may be the cause of the rise in glycoconjugate levels in plasma.

Serum sialic acid elevations are specific to tumour development. The size of the malignant tumour and the TSA levels were found to be positively correlated. TSA levels were elevated in lung cancer, according to (Kakari et al., 1988), who also stated that TSA is a more sensitive marker for the disease (Shasikanth MC et al., 1994)17 in line with (Plucinsky et al., 1986) proposed the role of total sialic acid in the development of disease. Serum sialic acid estimation's utility as a predictive marker for tumour growth is evident. According to research by Erbil KM et al. (1986), TSA is crucial for the staging, prognosis, and early identification of cancerous illness recurrence. Glycoconjugates are crucial for metastasis and invasion. Compared to cells with low metastatic potential, those with strong metastatic potential exhibited 80% more neuraminidase-susceptible sialic acid on their surfaces (Patel PS et al., 1990). Additionally, our findings showed that increases in LSA and PBH levels can serve as a precursor to malignant alterations. Serum fucose level (SFL) in cancer patients was found to be significantly changed in metastasis and advanced stage in earlier research, suggesting that it may be utilized as a predictive tool.

It's interesting to note that, in this study, patients with oral precancerous lesions had significantly higher amounts of glycoconjugates ( $p \leq 0.001$ ) than controls. Its significance as a diagnostic marker is increased by this distinguishing characteristic. They could be helpful as indicators to distinguish between oral cancer and precancerous lesions in the mouth. Patients with oral precancerous conditions and oral cancer may benefit from routine monitoring for glycoconjugates, which can help diagnose malignancy early on and enable treatment.

According to our findings, biomarkers such as TSA, PBH, and fucose have demonstrated promise in the detection of individuals with oral precancerous lesions and oral cancer. Analysing these markers may also aid in the late-stage detection of metastatic cancer, for which SFL and TSA may offer practical biochemical indicators for the clinical evaluation of the disease's invasiveness and dissemination in oral cavity malignancy. More case-controlled research on sizable populations utilizing these biomarkers will yield more empirical evidence that can be used to treat oral cancer.

## VI. CONCLUSION

In conclusion, the goal of the current investigation was to determine the serum glycoprotein levels in patients with oral cancer and precancer in comparison to controls. After running the ANOVA test on the study's data, statistically significant findings on the relationship between serum glycoproteins and oral precancer and oral cancer were discovered.

From protein estimation, it was discovered that all three parameters—serum total sialic acid, serum fucose, and serum protein-bound hexose were elevated. The serum protein bound hexose was substantially higher among the three glycoproteins. To obtain more scientific evidence that is helpful in the early identification of oral cancer, case control research involving a broader and diverse population can be conducted on the blood levels of Fucose, Total Sialic Acid, and Protein-bound Hexose.

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