Original Research Report

# Glycomic Analysis of Tear and Saliva in Ocular Rosacea Patients: The Search for a Biomarker

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**ABSTRACT** The purpose of this study was to study changes in glycosylation in tear and saliva obtained from control and ocular rosacea patients in order to identify potential biomarkers for rosacea. Tear fluid was collected from 51 subjects (28 healthy controls and 23 patients with ocular rosacea). Saliva was collected from 42 of the same subjects (25 controls and 17 patients). Pooled and individual samples were examined to determine overall glycan profiles and individual variations in glycosylation. O-and N- glycans were released from both patients and control subjects. Released glycans were purified and enriched by solid-phase extraction (SPE) with graphitized carbon. Glycans were eluted based on glycan size and polarity. SPE fractions were then analyzed by high-resolution mass spectrometry. Glycan compositions were assigned by accurate masses. Their structures were further elucidated by tandem mass spectrometric using collision-induced dissociation (CID), and specific linkage information was obtained by exoglycosidase digestion. N- and O-glycans were released from 20-μL samples without protein identification, separation, and purification. Approximately 50 N-glycans and 70 O-glycans were globally profiled by mass spectrometry. Most N-glycans were highly fucosylated, while O-glycans were sulfated. Normal tear fluid and saliva contain highly fucosylated glycans. The numbers of sulfated glycans were dramatically increased in tear and saliva of rosacea patients compared to controls. Glycans found in tear and saliva from roseatic patients present highly quantitative similarity. The abundance of highly fucosylated N-glycans in the control samples and sulfated O-glycans in ocular rosacea patient samples may lead to the discovery of an objective diagnostic marker for the disease.

**KEY WORDS** biomarkers, fucosylation, glycans, glycomic analysis, mucin, ocular rosacea, oligosaccharides, tear, saliva

## INTRODUCTION

Rosacea is a widely prevalent, chronic cutaneous disorder characterized by transient or persistent central facial erythema, telangiectasia, papules, pustules, and sebaceous gland hypertrophy localized primarily on the convexities of the central face (cheeks, chin, nose and forehead). This condition affects 13 million Americans and has been reported to have a prevalence of 10% in Sweden. It is most frequently observed in fair-skinned patients; however, Asians and African Americans have also been diagnosed with this disorder. It may occur both in men and women, at any age, but the onset typically begins after age 30.[1]

Ocular rosacea represents a common subset of the disorder and is often the cause of chronic inflammatory eye disease. While up to 90% of patients with ocular rosacea may have subtle roseatic skin changes, in 20% of cases, the ocular signs precede characteristic skin involvement, making the diagnosis of ocular rosacea particularly challenging in these patients.[2]

Unfortunately, there is no diagnostic test for either cutaneous or ocular rosacea. No specific histologic or serologic markers have been established to date. Biochemical methods for the early and accurate detection of ocular rosacea could potentially provide both a diagnostic marker as well as an etiologic explanation. Identifying a specific marker for the disease will enable earlier and more effective treatment.

The search for a biomarker is a long and difficult process, involving the identification of a biomolecule that is both sensitive and specific to a disorder. First, the

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**OUTLINE**



identification of the marker has to be reproducible in the population of interest (for example, rosacea patients). Second, testing in other populations (eg, non-roseatic blepharitis patients) is warranted in order to certify that the marker is specific to the condition. Additionally, different laboratories have to be capable of reproducing the results. This study is only the initial step in the long pathway toward the discovery of a biomarker for rosacea.

One of the major components of the tear film is mucin, which is a high-molecular-weight glycoprotein composed of tandem repeats of amino acids rich in serine (**Ser**) and threonine (**Thr**), which serve as sites for glycosylation.[3,4] Glycosylation is the most common post-translational modification process by which saccharides are linked to lipids and proteins.[4] Glycans attached to proteins in animal cells can be N-linked (bound to an asparagine side chain in an Asn-X-Thr or Asn-X-Ser amino acid consensus sequence with X ≠ proline), O-linked (with glycans attached to serine or threonine side chains) or glycosaminoglycans (bound to a serine side chain).[4,5] Oligosaccharides found in mucins are composed of primarily O-linked oligosaccharides, but also of N-linked glycans. Fucosylation, a type of glycosylation, comprises the attachment of a fucose residue to N-glycans, O-glycans, and glycolipids. Sulfation is a post-translational modification process in which a sulfate group ($SO_3H$) is attached to glycans. A review on mucins found in human ocular surface epithelia has been published.[6]

Our study is based on the fact that glycosylation is highly sensitive to the biochemical environment and has been implicated in many diseases.[7,27] Saliva and tear are biological fluids that are readily accessible and act as a mirror that reflects the levels of natural and artificial substances in the body. However, the glycosylation of tear and saliva has not been well studied. In this study, we globally profiled glycans isolated from tear and saliva of controls and ocular rosacea patients.

We have previously shown a high abundance of O-linked oligosaccharides in the tear fluid of patients with rosacea.[8] To the best of our knowledge, there are no reported studies of saliva in rosacea patients.

The objective of the present study was to perform glycomic analysis of tears and saliva of roseatic patients and compare them to normal patients in order to identify potential oligosaccharide markers for rosacea and to make an early and specific diagnosis possible. This may also enhance our understanding of this common and troublesome disease.

## MATERIALS AND METHODS

### Patient Selection

Tear fluid samples were collected from two groups of patients. Subjects with no ocular diseases comprised the control group. The test group consisted of patients with ocular rosacea. The diagnosis of ocular rosacea was based on the standard classification proposed by the National Rosacea Society Expert Committee.[9] According to these criteria, the presence of one or more of the following signs with an axial facial distribution is indicative of rosacea: flushing or transient erythema, persistent erythema, papules and pustules, telangiectasia. Patients with epithelial defects and/or corneal ulcers/infiltrates and history of recent (<1 year) ocular surgery were not included. This research followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board (IRB)/Ethics Committee. Informed consent was obtained from all subjects.

### Tear Sample Collection

Tear fluid from the inferior tear meniscus of 51 subjects (23 patients with ocular rosacea and 28 controls with no ocular diseases) was collected after light stimulation, using 10-μL microcapillary tubes (Microcaps, Drummond Scientific Co, Broomall, PA). The method of tear sample collection had been previously described by our group[8]; however, for this study we used a slightly modified technique, as follows. 1) Under the slit lamp, a bright light was shone onto the patients' eyes to stimulate tearing. 2) A microcapillary tube was held horizontally and its tip placed to touch the tear meniscus until the tube was completely filled or until the tear column inside the tube was no longer advancing. 3) The procedure was repeated in both eyes, and samples were transferred to a labeled microcentrifuge tube. 4) The samples were immediately frozen at −80°C until ready for biochemical analysis. During collection, the tip of the tube did not touch the eyelid or eyelashes. The patients were required not to instill eye drops of any kind for at least 1 hour prior to tear collection.

### Saliva Sample Collection

Saliva was collected from 42 of the same subjects (17 ocular rosacea patients and 25 controls). Samples were obtained with the aid of disposable plastic pipettes (Samco Scientific, San Fernando, CA) and were collected by the following method. 1) Patients were asked to collect saliva under their tongue. 2) Sublingual saliva was drawn into the pipette by gentle suction. 3) The saliva sample was transferred from the pipette into a labeled microcentrifuge tube. 4) Immediately after the collection, the samples were frozen at −80°C until biochemical analysis. The subjects were