Original Article

A study to assess serum and tissue levels of sialic acid and hexosamine in keloids

Girija Menon M[1], Moinuddin SK[2], Sampath Kumar V[3], Farhatunnisa[4]

[1]Associate professor, [2]Tutor, [3]Professor & HOD, Department of Biochemistry, Malla Reddy Institute of Medical Sciences, Hyderabad

[4]Professor, Department of Biochemistry, Gandhi Medical College, Hyderabad

Corresponding Author:
Dr. Girija Menon M
Email: drgirijamenon@gmail.com

Abstract:

Background: A keloid is an abnormal proliferation of scar tissue that forms at the site of cutaneous injury. The major component of scar tissue is the fibrous protein collagen, which is a glycoprotein. Sialic acid is a charged sugar that plays a key structural role in glycoprotein biosynthesis.

Objectives: Estimation of serum hexosamine and sialic acid in subjects with recurrent keloid formation as well as in normal subjects for comparison and to evaluate the role of these complex proteins in keloid formation.

Methods: A total number of 30 cases diagnosed as keloid and 30 controls attending to OPD were involved in the present study. The blood and skin biopsy sample were collected for estimation of biochemical parameters hexosamine and sialic acid.

Result: Serum levels of hexosamine (101 +/- 19.15) and sialic acid (1.435 +/- 0.34) in keloid subjects are not elevated significantly as compared to normal subjects (Serum Hexosamine 95.73 +/- 23.09) (Sialic acid 1.483 +/- 0.40) but significant ($p<0.0001$) increase in the tissue levels of sialic acid (0.98+/- 0.108) and hexosamine (1.66 +/- 0.419)are observed in keloid subjects as compared to normal (Sialic acid 0.65+/- 0.059 hexosamine 0.6+/- 0.109).

Conclusion: There is a significant increase in the levels of sialic acid and hexosamine in keloid tissue due to excessive deposition of collagen and matrix.

Key words: Keloids, Sialic Acid**,** Hexosamine

INTRODUCTION:

Keloid is a benign proliferative growth of dermal collagen due to excessive tissue response to skin trauma in pre-disposed individuals. Keloids can develop up to one year after injury. Keloids are cutaneous lesions composed of excess accumulation of extra cellular matrix. Keloid scars are characterized by over production of extra cellular matrix collagen and proteoglycan. [1] The process of tissue repair requires the coordinated completion of a variety of cellular activities including phagocytosis, chemotaxis, mitogenesis, and synthesis of collagen and extra cellular matrix components. In certain circumstances, the cellular processes that contribute to repair become unregulated leading to excessive scarring in the form of Keloids. [2] Successful healing requires the migration of mesenchymal cells into wound. Stimulated by growth factors, fibroblasts migrate into the wound through the extra cellular matrix. 5 to 7 days after wounding fibroblasts begin synthesizing collagen which increases in a linear fashion. Fibroblasts also produce zymogens that, when activated on the extra cellular matrix to enzymes, degrade newly synthesized collagen and glycosaminoglycans, and in doing so maintain a matrix balance between synthesis and degradation processes. Chronic inflammation is a common cause of excessive scar formation [3] Keloid fibroblasts manifest a loss of normal feedback in the regulation of extra cellular matrix production. They respond more vigorously to the growth factors. Biglycan/PG-I and decorin/PG-I are two small proteoglycans that are structurally related the altered

expression of biglycan in keloid tissue might be involved in the abnormal regulation of the extra cellular matrix [4]

The majority of mucopolysaccharides of connective tissues are un-branched polymers composed of disaccharide units typical for the species. The disaccharide repeating units are linked via the hexosaminidic bond to the hexuronidic group of the next following disaccharide. The hexosaminyl groups on the sulphate mucopolysaccharides are esterified with sulphate. The hexosamine of hyaluronate is D glucosamine, in chondroitin 4 & 6 sulphate and in dermatan sulphate, it is D galactosamine [5] Sialic Acid is a group of naturally occurring substances widely distributed in tissues like mucopolysaccharides linked with a sugar by a glycosidic bond at carbon 2 of the sialic acid. N acetyl neuraminic acid is an example of a sialic acid. These compounds are usually found as the terminal carbohydrate residues of oligosaccharides side chains of glycoproteins. Sialic acid is thus a collective term referring to various derivatives of neuraminic acid which chemically is a 9 carbon aldol condensation product of pyruvic acid with either D glucosamine or D mannosamine. In each of these sugars a hydroxyl group of the precursor has been replaced by an amino sugar. The amino groups are almost always acetylated. [6]. A key regulatory step in sialic acid synthesis is the formation of N acetyl mannosamine (manNAc) from UDP-N Acetyl glucosamine, catalyzed by uridine diphosphate N Acetyl glucosamine 2 epimerase.[7] Hexosamines are important components of the carbohydrate which are widely distributed throughout the body as a part of the structural elements of the tissues. The hexosamines like D-glucosamine, D-mannosamine and D-galactosamine as their acetyl derivatives are important constitutents of the mucopolysaccharides of tissues and other substances. [8]

In this study an approach is made to compare the levels of sialic acid & hexosamine in the normal & keloid tissues as well as in the serum of normal & keloid subjects in order to establish the role of these parameters in the repair mechanism of wound healing.

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MATERIAL AND METHODS

SELECTION OF CASES:

A total of 30 cases & 30 controls both male & female in the age group of 15-40 years attending to the plastic surgery outpatient department of Gandhi Hospital, Secunderabad were included in this study.

Inclusion criteria: Clinically diagnosed subjects with Keloid in the age group 15 - 40

Exclusion criteria: Subjects with hypertrophic scars and keloid cases on steroids

COLLECTION OF SAMPLE: Both serum & tissue samples were collected after approval from the institutional ethical committee.

SERUM SAMPLE: About 5 ml of blood was drawn using disposable syringes and collected in clean & dry plain bottles. In all cases care was taken to prevent haemolysis. Blood was allowed to clot and serum was separated by centrifuge. The sample was analysed for the biochemical parameters i.e. sialic acid and hexosamine.

TISSUE SAMPLE COLLECTION:

For the tissue studies keloid tissue from various sites were selected like ear lobe, sternal region and wrist area. So also keloids that developed following electricals burns, chemical burns, post surgical, post vaccination and those that developed spontaneously were all included in the study.

To estimate Sialic acid, under local anaesthesia and taking aseptic precaution, a biopsy of keloid tissue was taken. The tissue was weighed and then homogenized in 0.1 N sulphuric acid and then hydrolysed for 2 hours. 0.5ml of the hydrolysate was taken and sialic acid was estimated using the same procedure as serum. the results are expressed in micromoles / gm wet weight of tissue. Estimation of sialic acid was done by Thiobarbituric acid assay [9].

To estimate Hexosamine, under local anaesthesia and taking aseptic precaution, a biopsy of keloid tissue was taken. The tissue was weighed and then homogenized in 10ml of alcohol. It was then hydrolysed using 2ml of 3N hydrochloric acid for 4 hours. The hydrolysate was then treated just like serum for measurement of hexosamine levels. The results are expressed in mg/ gm wet weight of tissue. Hexosamine was estimated by: Modified Elson & Morgan method [10].

Similarly, normal skin samples were also collected and estimated for sialic acid and hexosamine.

RESULTS & DISCUSSION

60 subjects including cases & controls were studied for serum & tissue levels of sialic acid & hexosamine. Total cases were divided into two groups

Group I - normal subjects in the age group of 15 to 40 years
Group II - Keloid cases in the age group of 15 to 40 years

Table 1 shows the comparison between estimated levels of serum sialic acid in group I (normals) and group II (keloids cases). The serum sialic acid in group I are in the range of 1.408+/-0.400 mmol/lit of serum and that of group II is 1.435+/-0.340 mmol/litre of serum. Table I also shows the comparison between estimated levels of serum hexosamines in group I (normals) and in group II (keloids cases). It is seen that serum hexosamines in group I is in the range of 95.73+/- 23.09 mg/100ml of serum and in group II is 101+/-19.15mg/100ml of serum.

Table 2 shows comparision between tissue levels of sialic acid in group I (normals) and group II (keloid cases).The tissue levels of sialic acid in group I is seen to be in the range of 0.65+/- 0.059 micro moles/gm wet weight of tissue and in group II is 0.98 +/- 0.108 micro moles/gm wet weight of tissue.Table II also Shows comparison between estimated tissue levels of hexosamine in group I (normals) and in group II (keloid cases). Here it is seen that the tissue levels of hexosamine in group I is in the range of 0.60+/- 0.109

mg/gm wet weight of tissue and that of group II is 1.66+/- 0.419 mg/gm wet weight of tissue.

Serum levels of sialic acid and hexosamine in group I (normals) and in group II (keloid cases) are compared and the 'p' Values calculated and found to be not significant. Similarly comparison between tissue levels of sialic acid and hexosamine in group I (normals) and group II (keloid cases), with 'p' Values calculated was done and found to be statistically significant (<0.0001).

DISCUSSION:

The transformation of a wound clot into granulation tissue requires matrix degradation and biosynthesis that are balanced to achieve optimal wound healing. The degradation of extra cellular matrix is through the action of collagenase, proteoglycanases, and other proteases, which are released by mast cells, macrophages, endothelial cells, and fibroblasts. Importantly, either excessive synthesis of collagens, fibronectin, and proteoglycans by fibroblasts or deficient matrix degradation and remodeling may lead to abnormal lesions such as keloids.

It is seen from the results in table I that the serum sialic acid in normals is in the range of 1.483 +/- 0.400 m mol/litre of serum and that of keloids 1.435+/-0.340 m mol / litre of serum. Though there is an increase in the level of sialic acid in keloid cases, the 'p' value is not statistically significant. In the serum levels studies of hexosamines shown in table I in normals and keloid cases, the serum levels of hexosamine in normals is seen to be in the range of 95.73+/-23.099 mg / 100ml of serum while that of keloid cases is 101+/- 19.15 mg/100ml of serum. Here again through there is an increase in the hexosamine levels; the 'p' value is not statistically significant. This suggests that lack of collagen degradation may be resulting in excessive deposition and keloid formation. An increase in prolylhydroxylase activity has also been reported (Su C.W. et al 1998)[3].

Table II shows studies of the tissue levels of sialic acid in normals and keloid cases. Here we see that the sialic acid level in normal skin is 0.65+/-0.059 micor moles / gm wet weight of tissue and that of keloid cases is 0.98+/- 0.108 micro moles / gm wet weight of tissue. it is evident from the table that there is an increased level of sialic acid in keloid tissue as compared to the tissue in normals. The 'p' value is statistically significant.(<0.0001) This in accordance with the findings reported by (Tredget. E.E. et al 1997)[11], which showed that extraction of tissue samples from normal skin, hypertrophic scars and keloids has revealed an increased water content, and increased uronic acid (a component of all glycosaminoglycans except keratan sulphate), increased hexosamines, hexoses and sialic acid in keloids.

Type specific analysis of proteoglycans showed that decorin, a small molecular weight proteoglycan, was only 25% that of normal skin, yet the large chondroitin sulphate, proteoglycan, versican and biglycan were six fold higher. (Scott. P.G. Dodd. C.M et al 1996)[12] Similar results are also reported by (Balaba Tia et al 1972)[13] in their study on neuraminic acid levels in scar tissue of patients with keloids.

Comparison between the tissue levels of hexosamine in normals and keloids has also been reported by (Tredget E.E. et al 1997)[11]. In the present study, the normal tissue hexosamine levels is 0.60 +/- 0.109 mg/gm wet weight of tissue and the keloid tissue hexosamine level is 1.66 +/- 0.419 mg/ gm wet weight of tissue (Table II). The value of "p" is less than 0.001, which is statistically highly significant. Hence it is evident that there is an increased level of hexosamine in keloid tissue as compared to normal tissue. Results of the study are also supported by the observations of (Merkur' Eva etal 1969)[14]. In their study where in biochemical studies on hexosamines in keloid scars of patients were performed, an increased accumulation of hexosamine was found in keloid tissue as compared to the controlled levels in the normal skin. Excessive biosynthesis of extra cellular matrix proteins by fibroblasts has been proposed as one of the potential contributing factors to the accumulation of the excessive matrix. (Chau. D. et al 1998)[15].

Results of the study suggest that among the various parameters that have been studied in relation to the pathogenesis and treatment aspects of keloids, biochemical parameters like sialic acid and hexosamine also have an important role being components of the proteoglycans. However further studies of these parameters are required to find ways and means to regulate their excessive production along with that of other extra cellular matrix components so that treatment of keloid cases can give more positive results.

CONCLUSION:

In the present study, we observed that there is a significant increase in the levels of sialic acid and hexosamine in keloid tissue due to excessive deposition of collagen and matrix. Even though pathogenesis for the keloid formation is not clearly established, it is indicating that there is over production of glycosaminoglycans like sialic acid & hexosamine in the keloid scars.

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Table 1: Serum levels of sialic acid and hexosamine in normal skin and keloids

Serum Sialic acid (m mol /liter)	Normal skin mean+/- SD	Keloid mean +/- SD	P Values
	1.483 +/- 0.40	1.435 +/- 0.34	Not Significant
Serum Hexosamine (mg/100ml)	95.73 +/- 23.09	101 +/- 19.15	Not Significant

Table 2: tissue levels of sialic acid and hexosamine in Normal skin and keloids

	Group I	Group II	
Tissue sialic acid (micro moles/gm wet wt. of tissue)	Normal skin mean+/- SD	Keloid mean +/- SD	P Values
	0.65+/- 0.059	0.98+/- 0.108	<0.0001
Tissue Hexosamine (mg/gm wet. Wt of tissue)	0.6+/- 0.109	1.66+/- 0.419	<0.0001

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