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Determination of Molecular Weight of Keratin in Human Epidermis of Normal and Psoriatic Patients By Gel Filtration

S. M. MAHESAR[1], M. U. DAHOT[2] and M. Y. KHUHAWAR[3]

[1]Department of Biochemistry, Liaquat University of Medical and Health Sciences, Jamshoro
[2] Institute of Biotechnology and Genetic Engineering,
[3] High-Technology Central Resources, Laboratories University of Sindh, Jamshoro, Pakistan

*Corresponding Author: S. M. MAHESAR, E-mail: smmahesar@gmail.com , Cell# 0923013556757

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Abstract: The molecular weights of protein polypeptides obtained from normal and psoriatic skin samples after purification and isolation of their fractions were determined. The polypeptide chains compositions in human epidermis have shown that characteristic modifications of fibrous proteins occur during normal epidermal keratinisation. The polypeptide chains compositions in psoriatic scales differ from that of normal stratum corneum. The difference in between normal and psoriasis involved skin is probably due to the defect in synthesis of alpha- chain.

The epithelial protein fractions with low molecular weight keratins in epidermis was determined by Gel filtraration methods. Chromatography of these fractions to ascertain the accuracy and quantification of such proteins revealed the severity of psoriasis as inflammatory diseases. The protein markers of various molecular weights were used as standards. The keratin pattern of a given epithelial tissue may be modulated to some extent in the course of reactive changes, frequently resulting in higher complexity of keratin composition. In normal and psoriatic male and female subjects the molecular weight of isolated protein fractions ranged as 66 KD, 73KD, 68 KD and 69 KD respectively. Average molecular weight of purified protein fractions in normal and psoriatic male and female subjects was 60 ± 4, 44 ± 6, 52 ± 4 and 46±33 respectively. It has been noted that the a variations occurred in both of the genders of normal and psoriatic patients.

Keywords: Keratin Molecular weight. Psoriasis, Gel filtration. Epidermis.

INTRODUCTION

Most eukaryotic cells contain intermediate filaments (IF) of ~10 nm in diameter in their cytoplasm named as cytoskeletal system. The variety of keratins is outstanding due to its high molecular diversity in various components of Intermediate filaments proteins (Gonsebatt *et al.,* 2007). 54 functional genes consisting highest number in humans is reported by Roland Moll (Markus Divo and Lutz Langbein. 2008). The bovine muzzle ("prekeratin") reacts with inner organs and the polymerization and structural changes take place in keratin by dialysis. (Moll *et al.,* (2008). Mammalian has different molecular weight keratins as compare to other species (Roland and Langbein 1997). The Human keratin polypeptides are measured with different techniques including peptide mapping, Gel Electrophoresis and by use of western blotting methods. (Steiner 1978). The range of keratins in Human epidermis was measured ~44 to ~66 KD. (Jan Schröder and Georgyi 2009). The mechanical functions of epidermal Keratins can be experienced through inherited disease causes psoriasis in Humans (Diaz and Sneige 2007). The gene mutations in inherited disease may cause abnormalities that showing the blistering like structures on the skin surface (Fox K and Salzberg 2008). K_5 or K_{14} genes disturbances may cause a wide range of epidermolysis Bulosa simplex (EBS) or signs of skin destructions (Friederichs and Siewert 2007). The number of defected genes with epithelial and hair keratin have been identified upto nineteen mutant keratins (Galarneau and arceau 2007). The use of standards antibodies for the analysis of immunohistochemical reactions for the diagnosis of cancer and tumor have been frequently applied. Gu LH, Coulombe PA (2007). In cryptogenic liver cirrhosis, chronic pancreatitis and inflammatory bowel disease, the genes K_8 and K_{18} have been found defected. (Jackson 1980 and Kim 2006). A number of alcoholic liver diseases such as Mallory–Denk bodies develops due to the aggregations of stress proteins with altered K_8 and K_{18} proteins to found a new compound ubiquitin and p62 (Ku NO *et al.,*2003). Co relation of breast carcinoma with the level of K_8 and K_{18} proteins

noted through immune staining diagnosis for these patients. (Lane 2004 and Langbein 2008). The present work examines the molecular mass of keortain protein executed from psoriatic male and female patients and compared with male and female normal projects.

MATERIALS AND METHODS

We used marker protein as standards for the determination of the molecular weight of purified proteins extracted from the skin of normal and psoriatic human subjects. Molecular weights may be determined from osmotic pressure measurements. The procedure is most successful for molecular weights from about 30 KD to 100 KD because some of the smaller molecules may leak through the membranes used while the very large molecules gave a very low osmotic pressure relative to the weight concentration. The protein markers, alcohol dehydrogenase, albumin (Bovine) serum, carbonic anhydrase and cytochrome-c (Sigma. U.S.A) were purchased from local market. The molecular weight of purified protein fractions of human epidermis of normal male and psoriatic male (n=40) each and normal and psoriatic female subjects (n=31) were determined by comparing with marker protein standards. The samples were prepared by keratin extraction methods [18-19] and the reagents we prepared 0.1M HCl (9 ml of HCl diluted to 1000 ml DDW), 0.1M TRIS solution 12.11gm in 1000 ml DDW) and 0.05M 2-Mercaptoethanol (Me) solution (Diluted 3.9 ml of mercaptoethanol to 1000 ml DDW). The working solution for the extraction of keratins we prepared, Tris-HCL buffer (pH 9.0) by taking, HCl-Tris-Urea-Me in proportion of (40ml, 500ml, 480gm and 90ml respectively). The ingredients of working solution were mixed well and adjusted the pH to 9.0 the volume was made up to 1000 ml DDW. Biopsies of skin samples weighing (0.76g) previously defatted dried and cut in to slices was suspended in 200ml of working solution in 500ml volumetric flask and was kept on magnetic stirrer with magnetic bar. The sample suspended was continuously rotated for three hours then the contents were centrifuged for 20 minutes at 8000xg in refrigerated centrifuge. The supernatant was preserved as extracted protein sample for further analysis.

Determination of molecular weight: Proteins having high molecular weights do not enter the gel pores, but pass through the fluid volume of a column of porous gel particles faster than those having low molecular weight. Molecular weight determination of unknown proteins ratio of v_e/v_o compared to the v_e/v_o of protein standards of known molecular weight with the elution volume and the void volume was used. The void volume of a given column based on the volume of effluent required for the elution of a large molecule such as Blue Dextrin-2000 (molecular weight approx. $2x10^6$). A calibration curve was prepared by plotting the logarithms of the known molecular weights of protein standards versus their respective v_e/v_o values.

Preparation of Gel: Sephadex (Anhydro glucose polymer cross linked with epichlorohydrin). Sephadex G-100, 25 Gm mixed with 250 ml of purified water and left over night for completing the hydration. On the next day the upper layer of the water was removed and remixed with purified water and by putting magnetic bar in to the gel rotated for ten minutes. The excess water was removed and washed with Tris buffer and finally with double distilled water.

Procedure: The slurry was washed 3 times till removal of fines and degassed for 10 minutes by placing it under vacuum for removal of air bubbles. A cotton plug was inserted at the end of vertically placed column. 40-ml buffer poured in to the column and allowed 20-30 ml to drain out the bottom before closing the out let. The gel slurry poured gently down the side of the column with the help of glass funnel and then the out let was opened. The gel was allowed to settle down to the required height and the remaining volume of the column was filled with buffer and placed an airtight cork on the column and connected with buffer reservoir. Two bed volumes allowed passing through the column for equilibration. Blue dextrin-2000 applied for determination the base volume of the column. The buffers were allowed draining until it reached the bed surface. The outlet and applied 10-ml of sample to the column surface carefully by the help of pipette and connected outlet tube with the fraction collector at fixed absorbance (280 nm). The sample was allowed to enter the gel through opening the outlet and 5ml of buffer was poured before the sample reached bed surface. Finally the column was connected with buffer reservoir collected the fractions and evaluated recorded result accordingly (McLean, 2007),(Putman,1959),(Lowry,1951),Christopher, 2008) (Bramhall, 1969) and (McGuire, 2006).

RESULTS AND DISCUSSIONS

The molecular weight of purified protein fractions determined from human epidermis of psoriatic male (n=40) and female subjects (n=31) compared with equal number of the normal control counter parts against the protein markers. The molecular weights of protein markers chromatographically measured as 150,000, 66,000, 29.000 and 12,400 Dalton **(Table–1)**. Average molecular weights of purified protein fractions in normal and psoriatic male and female subjects **(Fig-1)**, observed at 95% confidence limit were 60 ± 4

against 44 ± 6 and 52 ± 4 against 46 ± 3.3 KDa respectively. The difference in mean values clearly indicates that the molecular weights in psoriatic samples decrease due to keratinization disorder. Standard deviation calculated in normal/psoriatic male and female samples, 18/18.5 and 18/19 while coefficient of variation percent (CV %) was 0.2/0.9 and 0.6/0.7 respectively. The range of the molecular weight in normal/psoriatic samples calculated with SPSS version that stands 66 KD/ 73 KD and 68 KD/69 KD.

Table-1Molecular Weights of Marker protein standards

S. No	Protein Marker	Molecu-lar Weight, Daltons	Elution time	Mean of Time Factor	No. of Fractions
1	Alcohol Dehydro genase	150000, Da	6.33 Minutes	T.T= 1	(1)
2	Albumin (Bovine) Serum	66000, Da	15.75 Minutes	T.T= 2.5	(2)-(3)
3	Carbonic anhydrase	29000, Da	63.3 Minutes	T.T= 7	(4)-(10)
4	Cytochro me-C	12400, Da	80.6 Minutes	T.T= 12	(11)-(13)

T.T = Total time, Da = Dalton (unit of m/w

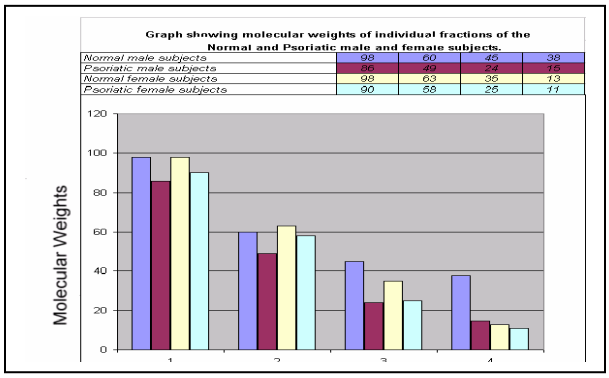

Fig-1. Graph showing Molecular weight individual factions of the normal and psoriatic male and female subjects.

The data obtained correlates with the other researchers internationally. Significant variation was found in normal and psoriatic samples **(Table-2)**. The molecular weights (mean) of psoriatic samples were blow as compared to normal samples. It was noted in a few cases that the number of protein fractions from normal extracts were up to five but with negligible amounts of last fraction while in psoriatic samples mostly produced up to four fractions. The protein fractions of normal male against psoriatic male samples differentiate by 98/86, 60/49, 45/24, 38/15KD while in normal female against psoriatic female samples distinguished as 98/90, 63/58, 35/25, 13/11 KD respectively. The ultra structure of filaments made protein from keratin of psoriasis scales were distinct as compared to the filaments obtained from normal epidermis. The appearance of keratin disorder leads to abnormality caused as hyper proliferation as reported. The Keratin polypeptide of psoriatic epidermis contains significant amount of 2 keratin polypeptides with molecular weight 56 and 50 KD while normal epidermis showed increased molecular weight 69,65.5 and 65.2.KD (Levine and McLeod 1979).The Keratins of normal proteins of normal and abnormal human epidermal keratin consists three chains molecular weight of 65 KD, 60, KD and 52, KD respectively (Katagata 1984). The differences were detected in electrophoretic pattern when compared with abnormal tissues. The modifications of human epidermal prekeratin in skin surface which was composed of 70 KD, 58KD, 56KD, 49KD, 50KD,55KD polypeptide and 63KD polypeptides were present in the skin of palm and sole, Bowden (1981). Keratin polypeptides in normal and psoriatic human epidermis profile observed (68, 60, 58, 52, 66, 58 and 55 KD) respectively. The quantity of keratin from involved psoriatic epidermis showed a variable reduction in the 68-KD polypeptide and an altered expression of smaller Mol. Weight 40 to 55 KD. Keratin from the psoriatic lesion was abnormal and appeared as a defective keratin in psoriasis. Svedberg of Sweden demonstrated that tris-urea-mercaptoethanol extractable proteins from human epidermis could be polymerized under similar conditions as used in other mammalian studies. It has noted that the larger keratins were basic and smaller keratins were acidic in nature. Amino acid analysis of these filaments of psoriasis differs from those of normal individualism. In injury at least two keratins may be the responsible for the creating cell proliferation abnormality markers of proliferation or in injury. Two keratin molecular weights of 50 KD and 56 KD in psoriatic epidermis synthesized to and produce keratinization disorder (Wantz, *et al.*, (1980).

Table-2, Molecular weights of purified protein fractions isolated from human skin of normal and psoriatic

Observations	Mean ± KDa	S.D	C.V	S.M.E	Max	Min	Range	P. Value
-Normal samples males (n=40)	60±4	18	0.22	2.8	94	26	66K.Da	>0.03
-Psoriatic samples males (n=40)	44±6	18	0.9	2.8	88	14	73K.D	>0.05
-Normal samples females (n=31)	52±4	18	0.7	3.2	90	22	68K.D	>0.05
-Psoriatic samples females (n=31)	46±3.3	19	0.6	3.5	78	21	69K.D	>0.05

Abbreviations: S.D= Standard deviation, C.V= Coefficient of variation, S.M.E= Standard mean error, K.D= Kilo Daltons, (mean ±) observed at 95% confidence limit .05+95% confidence limit

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