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In Silico Physico-Chemical Comparative Study of Human And Camel Insulin

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Abstract:-The Physical, Chemical, Conformational, And Energetic Properties Of Amino Acid Residues In Turn Alter The Physiological Properties Of The Activating Protein Molecules. The Sequence Comparison Of Insulin Chain A And Chain B Of *Camelus Dromedaries* Was Compared To *Homo Sapiens* . Although The Sequences Differed Only In Four Amino Acids But Their Physico-Chemical Properties Stretched Apart. Camel Milk Insulin Had Large Mutable Affinity For Alanine Which In Turn Is Responsible For –I. High Thermo-Stability Ii. High Aliphatic Index And Iii. High Hydropathicity. Due To This Altered Behavior Of Insulin Molecule In Camel, It Does Not Forms Coagulum And Is Resistant To Proteolysis During Digestion. In Gut Part This Indigestible Insulin Peptide Enters Circulatory System And Regulates Glycemic Loads In Diabetic Patients.

Keywords*: Aliphatic Index, Camel Milk, Flexibility, Grand Average Of Hydropathicity, Instability Index, Insulin, Polarity, Relative Mutability.*

I. INTRODUCTION

Camel Milk Forms The Nutritional Source Of Many Rural And Sub-Rural Populations Of Harsh And Arid Regions Of Asia. It Is Consumed In Its Fresh State And Storage Is Not Preferred Because Of Its Souring Properties. Ethnically It Is Believed To Be Super Functional Food For Its Protective And Remedial Actions To Various Ailments Among Which Diabetes Forms Its Prime Usage [1]. In Hyperglycemic Phases It Mimics Insulin As Its Therapeutic Peptides Are Resistant To Digestion Hence It Does Not Form Coagulum In A Mild Acidic Environment [2]. Such Specific Biochemical Attribute Are The Outcomes Of Low Degree Casein Phosphorylation[3]. It Reduces Post-Prandial Glucose And Hba1c, Auc-Insulin And Auc-Glucose Along With Homa-Ir. Many Clinical Trials Have Been Projected Since Times To Unearth The Basic Mechanism Of Camel Milk In Glycemic Regulation. Camel Milk Insulin Readily Enters Circulatory System Without Being Catabolized In Digestive System. The Possible Routes To Such Mechanism Include- I. Camel Milk Insulin Is Resistant To Proteolysis Ii. Camel Milk Insulin Is Encapsulated In Lipid Vesicles As Nanoparticles (Lipid Vesicles) Iii. Some Other Elements Of Camel Milk Make It Anti-Diabetic And Iv. Presence Of Small 'Insulin-Like' Molecules / Substances That Mimic Insulin Interaction With Its Receptor [4,5]. However, The Underlying Mechanism Is Yet Not Fully Understood. Among These Four Arrays A Present Study Was Carried Out To Explore The Phsico-Chemical Differences Between The Insulin Component Chains Between Camel And Humans To Evaluate The Non-Coagulum Properties Of Camel Milk. Polypeptides Are Highly Sensitive To Biochemical Milieu And Their Physico-Chemical Properties Largely Depend On Their Constituting Residues. The Tertiary Structure Of The Insulin Is Determined By The Specified Amino Acids Arranged In A Particular Loci And Pattern And It Has Been Invariably Conserved During Vertebrate's Molecular Evolution [6] . Amino Acid Residues At A1-A5, A19, A21, B12, B16 And B23-B26 Of Insulin Forms Receptor-Binding Domain [7]. During Molecular Evolution Amino Acid Were Substituted With Residues Of Same Chemical Groups With High Likelihoods. Certain Amino Acids With Similar Physicochemical Properties Can Be More Easily Substituted Than Those Without Similar Characteristics [8]. Substitutions With Similar Residues Preserve The Essential Structural And Functional Features. However, Substitutions By Residues Of Different Physico-Chemical Properties Are More Likely To Cause Disruptions In Function And Metabolic Processes [9, 10]. Understanding The Underneath Kinetics Through Physic Chemical Properties Will Help In Understanding The Therapeutic Mechanism Of Camel Milk In Diabetes And Its Associated Pathologies.

II. MATERIALS AND METHODS

1. *Protein Sequence Retrieval And Local Alignment* : Preproinsulin And Its Respective Component Sequences Were Retrieved From Uniprot (Http://Www.Uniprot.Org/) And Were Locally Aligned Using Blast Tool With Needleman-Wunsch Global Align.
2. *Analysis Of Physico-Chemical Properties*: The Expasy'S Protparam Tool (Http://Web.Expasy.Org/Protparam/) And Prot Scale (Http://Web.Expasy.Org/Protscale/) Was Used To Compute Amino Acid Composition (%), Molecular Weight, Theoretical Isoelectric Point, Number Of

Positively And Negatively Charged Residues, Instability Index, Aliphatic Index , Grand Average Of Hydropathy , Average Flexibility , Relative Mutability And Polarity.

III. RESULT AND DISCUSSION

In Silico Local Alignment Of Human Insulin And Camel Insulin Residues Reveals That Both Are Nearly Identical With Four Substitutions With Equally 2 Residual Substitutions In Both The Chain A And Chain B. 90 % Identity And 95 % Positives Were Obtained While Comparing Chain A While Chain B Had 93 % Both Identity And Positives .All The Three Primary Receptor-Binding Surface Of Insulin: (I) The N-Terminal And C-Terminal Segments Of The A Chain (**Gly** ^{A1} **-Ile** ^{A2} **-Val** ^{A3} **-Glu** ^{A4} And **Tyr** ^{A19} **-Cys** ^{A20} **-Asn** ^{A21}), (Ii) The Central A -Helix Of The B Chain (Especially **Val** ^{B12}) And (Iii) And The C-Terminal Segment Of B Chain (**Phe** ^{B24} **-Phe** ^{B25} **-Tyr** ^{B26}) Were Conserved In Camel Insulin As Akin To Human Insulin. These Surfaces Are Classified As Its "Site-1" Related Binding Surface In Relation To "Site-2" Related Surface. The Putative Site-2 Related Surface Of Insulin, Is Proposed To Correspond To Its Hexamer-Forming Surface Which Includes Residues **His** ^{B10} , **Leu** ^{B17} , **Val** ^{B18} , **Ser** ^{A12} , **Leu** ^{A13} And **Glu** ^{A17} . Substitutions In Any Of The Residues At Site 2 Affects The Kinetic Properties Of Hormone Binding Disproportionately To Effect On Its Affinity. Such Kinetic Properties (Related To The Residence Time Of The Hormone-Receptor Complex) Correlate With Relative Post-Receptor Signaling Pathways; Prolonged Residence Times Also Favors Mitogenic Signaling Relative To Metabolic Signaling. This "Site-2"In Camel Insulin Was Identical To That Of Human Insulin Pointing Similar Dimer And Hexamer Structural Affinities [11] (Fig.1). Physico-Chemical Comparsion Of Human And Camel Insulin Chain A And Chain B Reveal Identical Isoelectric Point, Total No Of Negative Charge Residue (Asp+ Glu) And Total No Of Positive Charge Residue (Arg +Lys). Aliphatic Index, Which Is Also Defined As The Relative Volume Of The Protein Occupied By Aliphatic Side Chains I.E. Alanine, Valine, Isoleucine, And Leucine Of Proteins Is Significantly Higher In Camel Chain B Than That Of Human Insulin Chain B. This Index Is A Positive Factor For The Increase Of Thermostability Of Globular Proteins [12]. Instability Index Was Similar For Chain B While For Chain A It Was Comparatively Lower In Camel Insulin Referring Its More Stability. The Stability Of Any Peptide Is A Result Of An Increased Alanine Residues Although It Does Not Affect The Secondary Structure [13,14] . Grand Average Of Hydropathicity Differed For Both The Chains In Both The Organisms. Gravy Was Higher In Both Insulin Component Chain A And Chain B Of *Camelus Dromedaries* As Compared To *Homo Sapiens*. In Both The Insulin Components Chain A Had More Hydrophobicity As Compared To Chain B (Table 1). Various Forces Are Involved In Unique Conformations Of Polypeptide Chains. This Resulting Pattern Allows Hydrophilic Side-Chains Access To The Aqueous Media While Consequently Minimizes Contact Between Water And Hydrophobic Side-Chains [15]. It Also Reveals The Extent Of Residual Depth In Tertiary Structures Signifying Hydrophobicity Of The Molecule [16]. The Structural Conformations Are The Result Of Both Hydrophobicity And Steric Effects That Determine Packing Between The Secondary Structures In The Crowded Interior Of The Macromolecule [17]. Minimum Polarity/Gantham Values And Relative Mutability For Insulin Molecule Were Similar In Both *Camelus Dromedaries* And *Homo Sapiens* While In Terms Of Maximum Value Camel Insulin Molecule Is More Polar And Mutable Than Human Insulin (Fig 2). The Relative Mutability Of Each Amino Acid Is Proportional To The Ratio Of Changes To Occurrences. In Camel Insulin Chain A And B The **Ala** Residue Is The Mutable Choice Directing Thermo-Stability To The Molecule [18,19].Average Flexibility Was Identical For Both Minimum And Maximum Values In Both The Studied Molecule. This Flexibility Is A Dynamic Character Of A Insulin Molecule. Among The Non-Polar Residues **Tyr** And **Ala** Fluctuate More Than The Others While In Polar Types **His** Tends To Be Less Flexible. The Bend-Forming Residues **Gly** And **Pro** Are Equally Flexibile Akin To Highly Flexible Polar Amino Acid Residues. The Residues **Lys**, **Asp** And **Ser** Of The Polar Group Are Exceptionally Flexible. The Buried Effect Of The Non-Polar Residues Is Indicates Less Flexible Trends Of **Phe**, **Met** And **Trp** [20].

IV. CONCLUSION

Ingestion Of Camel Milk As A Therapeutic Source In Diabetes Has Been Practiced In Various Parts Of The World. The Underneath Mechanism Of Its Intact Insulin During Digestion Process Is An Probable Outcome Of Alanine Incorporation In Camel Insulin During Molecular Evolutionary Process Due To Which It Does Not Forms The Coagulum And Leads To Resistance Of Insulin Molecule In Digestive Proteolysis. Alanine Increases Hydro-Pathcity And Aliphatic Index Due To Which The Insulin Has More Thermo-Stability Index And Hence It Enters The Circulatory System Without Degeneration. It Can Be One Of The Major Routes For Its Therapeutic Glycemic Load Regulation.

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Cd	F	A	N	Q	H	L	C	G	S	H	L	V	E	A	L	Y	L	V	C	G	E	R	G	F	F	Y	T	P	K	A
Hs	F	V	N	Q	H	L	C	G	S	H	L	V	E	A	L	Y	L	V	C	G	E	R	G	F	F	Y	T	P	K	T

Chain A

Cd	G	I	V	E	Q	C	C	A	S	V	C	S	L	Y	Q	L	E	N	Y	C	N
Hs	G	I	V	E	Q	C	C	T	S	I	C	S	L	Y	Q	L	E	N	Y	C	N

Table 1: *In Silico* Physico-Chemical Comparison Of Human And Camel Insulin Chain A And Chain B Using Prot Pram

S.No	Pcp	Chain A		Chain B	
		Hs	Cd	Hs	Cd
1	Naa	21	21	30	30
2	Mw	2383.71	2339.65	3429.96	3371.88
3	Pi	3.79	3.79	6.90	6.90
4	Tnnr (D + E)	2	2	2	2
5	Tnpr (R + K)	0	0	2	2
6	Ii	22.54**	7.02**	9.85**	9.85**
7	Ai	88.10	88.10	81.33	84.33
8	Gravy	0.214	0.319	0.220	0.223

1. Hs-*Homo Sapiens* And Cd-*Camelus Dromedarius*
2. Pcp-Physicochemical Parameter; Naa-Number Of Amino Acids; Mw-Molecular Weight ; Pi-Isoelectric Point; Tnnr (D + E) - Total Number Of Negatively Charged Residues (Asp + Glu); Tnpr (R + K) - Total Number Of Positively Charged Residues (Arg + Lys); Ii- Instability Index; Ai-Aliphatic Index And Gravy- Grand Average Of Hydropathicity
   *- Unstable; **- Stable

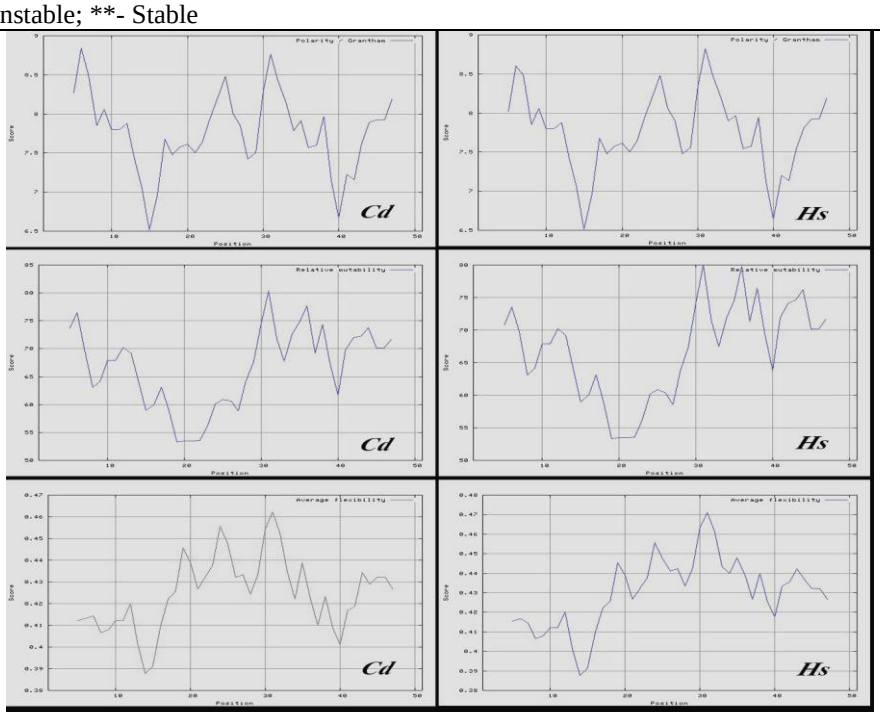

Figure 2- In silico comparison of polarity, relative mutability and average flexibility of insulin molecule in *Camelus dromedaries* (Cd) and *Homo sapiens* (Hs)