3D protein structure of CCL2, CCL5 and CXCL10 chemokines in multiple sclerosis

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3D protein structure of CCL2, CCL5 and CXCL10 chemokines in multiple sclerosis

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ABSTRACT

Background: When bone marrow-derived and resident cells migrate to sites of inflammation, chemokines and chemokine receptors play crucial roles. Multiple sclerosis immunological triggers and the therapeutic effects of systemic treatment can be learned from peripheral blood biomarkers. This work aimed to drew the 3D protein structures of chemokines (CCL2, CCL5 and CXCL10) and observe the changes in amino acid analysis.

Methods: A designable study was conducted on CCL2, CCL5 and CXCL10 chemokines that related to multiple sclerosis (MS) in Multiple Sclerosis Center during November 2021 to May 2022. 3D protein structure done by sequencing of CCL-2, CCL-5 and CXCL-10.

Results: 3D protein structure of CCL-2, CCL-5 and CXCL-10 showed a convergence between study CCL-2 isolated and that of the Gen-Bank database (NCBI-2024) with identities 19/20 (95%) CCL2 had a one mutations appeared as (D to E). While there a high convergence between study CCL5 isolated and that of the GenBank database (NCBI-2024) with identities 91/91 (100%). A low convergence between study CXCL10 isolated and that of the GenBank database (NCBI-2024) with identities 22/28 (79%) in CXCL10 had a six mutations noticed as (C to I), (R to I), (V to Y), (E to F), (I to Y), and (I to S).

Conclusion: No studies about chemokines of MS in Iraq, so the present study is found necessary as a first study to determine chemokines 3D protein structure among patients with MS in Iraq –generally- and in Basrah – especially.

Keywords: chemokines, CCL2, CCL5, CXCL10, 3D protein structure, convergence

INTRODUCTION

When bone marrow-derived and resident cells migrate to sites of inflammation, chemokines and chemokine receptors play crucial roles [1].

Chemokines have roles in immunological modulation, T cell polarization, stimulation of respiratory burst, apoptosis, angiogenesis, mitosis, tumor metastasis, wound healing, and release of cytokines and extracellular matrix proteases, in addition to supporting leukocyte recruitment. Gaining further understanding of lesion evolution, the pathophysiology of the disease, and the identification of possible therapeutic targets are the key draws of researching chemokines in multiple sclerosis. Nonetheless, it is still difficult to assign specific pathogenic roles for chemokines and their receptors in disorders of the human central nervous system [2].

They are a chemo-attractant cytokines, are a broad class of tiny, basic proteins that range in molecular weight from 8 to 14 kDa. They are distinguished by their ability to draw leukocytes to areas of infection and inflammation [3].

Yoshimura et al. (1987) discovered monocyte-derived neutrophil chemotactic factor (MDNCF), a putative modulator of the leukocyte-specific inflammatory response. More than 50 distinct chemokines have been found in humans as a result of the chemokine family's significant research since then [4].

Bio-markers are quantifiable normal biological and pathological processes indicators, as well as pharmacological reactions to interventions of therapies in multiple sclerosis. A prospective biomarker should be reliable and able to distinguish between healthy persons and multiple sclerosis patients. The use of biomarkers has traditionally been limited to identifying altered proteins in body fluids (blood, CSF, and urine) [5].

In the periventricular brain white matter and superficial spinal-cord, where CSF interacts with white matter, most MS lesions are detected [6]. Since brain biopsies are rarely accessible, CSF is a crucial material to comprehend MS pathophysiology. CSF can quantify a range of soluble indicators and cell populations using flow cytometry analysis, PCR research, and functional cell observations. CSF is typically taken during a diagnostic procedure, however because it is an intrusive technique, it is not often taken [5].

Multiple sclerosis immunological triggers and the therapeutic effects of systemic treatment can be learned from peripheral blood biomarkers [6]. It is also worth noting that the bulk of lumbar CSF-protein contents are sourced from blood, with the remaining consisting primarily of brain-deriveds or intra-thecally synthesized protein making up the remainder [7].

Chemokines can be categorized into five groups: CC (C-like chemokines), CXC (C-like chemokines), XC (C-like chemokines, often referred to as the C subfamily), CX3C (C-like chemokines), and CX chemokine [8]. These groups are based on the quantity and spacing of cysteine residues that are involved in the creation of disulfide bonds.

XC chemokines only have two cysteines, whereas the CC, CXC, and CX3C families of chemokines have four. Human chromosome 17 is home to a cluster of genes known as CC chemokines, which are the largest group of proteins with two adjacent cysteine residues close to their N-terminus. The majority of CXC chemokines are grouped on human chromosome 4, and the first two of the four cysteine residues are separated by one or three extra amino acids (designated 3X or X in their names) in the CX3C and CXC chemokine subfamily. Nomiyama (2008) discovered the fifth subfamily CX chemokine in zebrafish and found that it possesses the third and fourth cysteine residues intact while lacking one of the two N-terminal cysteine residues [9].

Multiple sclerosis (MS) is a chronic autoimmune inflammatory illness that is typified by demyelinating and neuro-degeneration. There is currently general agreement that key components of MS pathophysiology include the infiltration, accumulation, and activation of macrophages and T cells specific

to myelin in the central nervous system [10–12].
The primary mediators of this inflammatory process include chemokines, cytokines, CD4+ T cells, and chemokine receptors. Th1, Th2, and Th17 subsets of helper T cells can be distinguished from one another by their distinct cytokine production patterns and effector roles. Th1 cells primarily secrete TNF and IFN-μ and are in charge of cellular immunity [13].
Th2 cells have the ability to produce cytokines including IL-4, IL-5, and IL-10 and are frequently implicated in humoral immunity. Th17 cells are primarily responsible for the inflammatory response and secrete IL-17 and IL-6. The expression pattern of chemokine receptors would bestow upon every group a distinct attribute of movement to ligand chemokines. Numerous studies now underway have demonstrated the immunoregulatory role of chemokines and chemokine receptors in the pathogenesis of multiple sclerosis [14].
This work aimed to drew the 3D protein structures of chemokines (CCL-2, CCL-5 and CXCL-10) and observe the changes in amino acid analysis.

METHODS

Design and setting

A designable study was conducted on chemokines types (CCL-2, CCL-5 and CXCL-10) that related to multiple sclerosis (MS) in Multiple Sclerosis Center during November 2021 to May 2022.

3D Protein Structure

3D protein structure done by sequencing of CCL2, CCL5 and CXCL10.
It was done by comparison with preserved protein in Blast, NCBI-2024 and drawing by NCBI-2024.

Ethics

Approved by IRB committee of Department of Medicine, College of Medicine, University of Basrah (No.109/2021 [479] in 17/11/2021).

RESULTS

3D protein structure of CCL-2, CCL-5 and CXCL-10 showed a convergence between study CCL-2 isolated and that of the Gen-Bank database (NCBI-2024) with identities 19/20 (95%) CCL2 had a one mutations appeared as (D to E). While there a high convergence between study CCL-5 isolated and that of the GenBank database (NCBI-2024) with identities 91/91 (100%). A low convergence between study CXCL10 isolated and that of the GenBank database (NCBI) with identities 22/28 (79%) in CXCL10 had a six mutations seen as (C to I), (R to I), (V to Y), (E to F), (I to Y), and (I to S). No previous studies interested studied in 3D protein with chemokines, so we cannot discussed the current research.

3D protein structure for CCL2

BLASTX search protein databases using a translated nucleotide queryChain B, C-C motif chemokine 2
PDB: 4ZK9_B
GenPept Identical Proteins Graphics
The chemokine binding protein of orf virus complexed with CCL2 [Homosapiens]

Sequence ID: 4ZK9_BLength: 83 Matches Number: 1

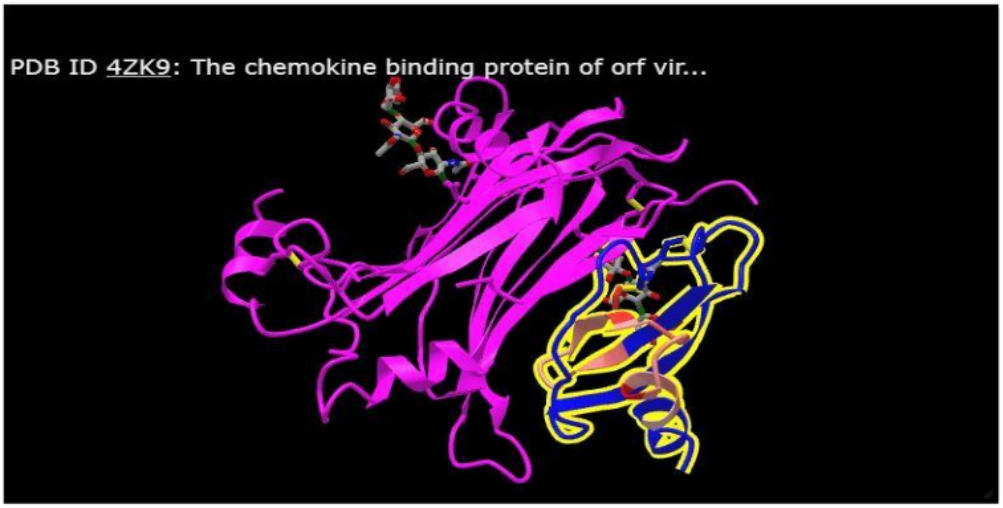
Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps	Frame
44.7 bits(104)	0.010	Compositional adjust.	matrix 19/20(95%)	20/20(100%)	0/20(0%)	+3

Query	393	FKTIVAKEICADPKQKWVQ 452 E FKTIVAKEICADPKQKWVQ +
Sbjct	43	FKTIVAKEICADPKQKWVQ 62 D

Query	393	FKTIVAKEICADPKQKWVQ 452 E
Sbjct	43	.....**D** 62

Figure 1. Chemical structure of CCL2-PE-DTPA complete.



https://www.ncbi.nlm.nih.gov/Structure/icn3d/full.html?from=blast&blast_rep_id=4ZK9_B&query_id=Query_81203&command=view+annotations;set+annotation+cdd;set+annotation+site;

set+view+detailed+view;select+chain+4ZK9_B;show+selection&log$=align&blast_rank=6&RID=93YAEJG2016

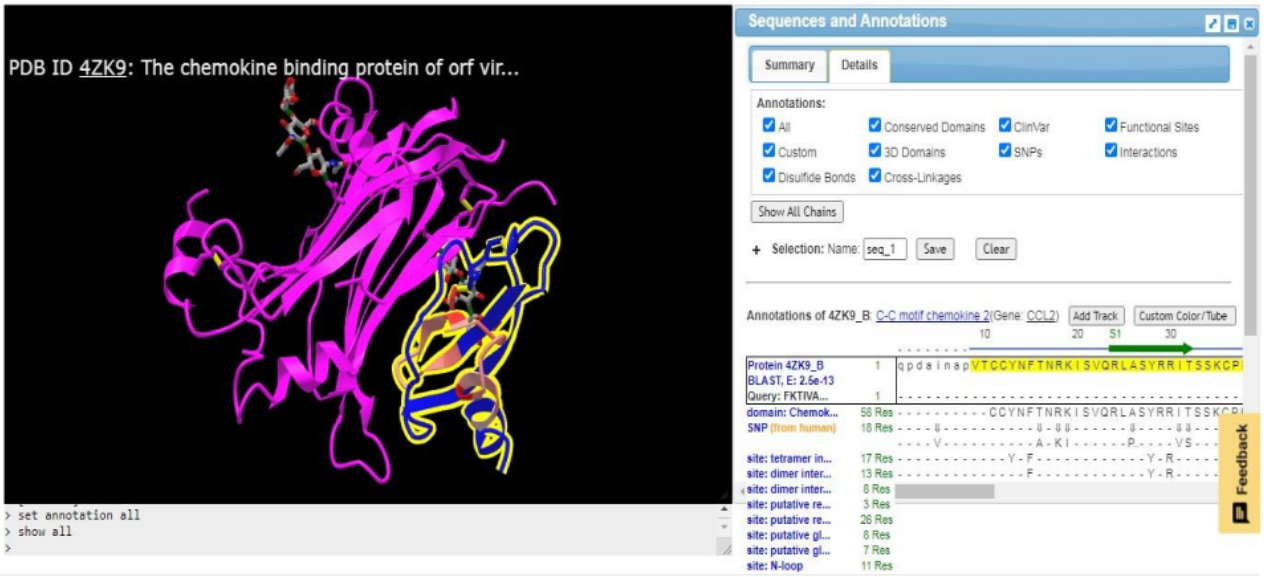


Figure 2. The chemokine binding protein of *orf virus complexed* with CCL2 [Homo sapiens].

Table 1. Showing wild-type and amino acid variation after DNA molecules exposure to SNP mutation at different locations.

Sample No.	Wild Types (Subjects)	Amino acid Variation (Query)
2	D (Aspartic acid)	E (Glutamic acid)

3D Protein Structure for CCL5

Chemokine (C-C motif) ligand 5 [Homo sapiens]GenBank: AAY22177.1

GenPept Identical Proteins Graphics

>AAY22177.1 chemokine (C-C motif) ligand 5 [Homo sapiens]
MKVSAAALAVILIATALCAPASASPYSSDTTPCCFAYIARPLPRAHIKEYFYTSGKCSNPAVVFVT
RKNRQVCANPEKKWVREYINSLEMS

C-C motif chemokine 5 isoform 1 precursor [Homo sapiens] Sequence ID: NP_002976.2Length:

91Number of Matches: 1 Gene-associated gene details

Genome Data Viewer-aligned genomic context Identical Proteins-Identical proteins to NP_002976.2

Range 1: 1 to 91GenPeptGraphics

Alignment statistics for match #1

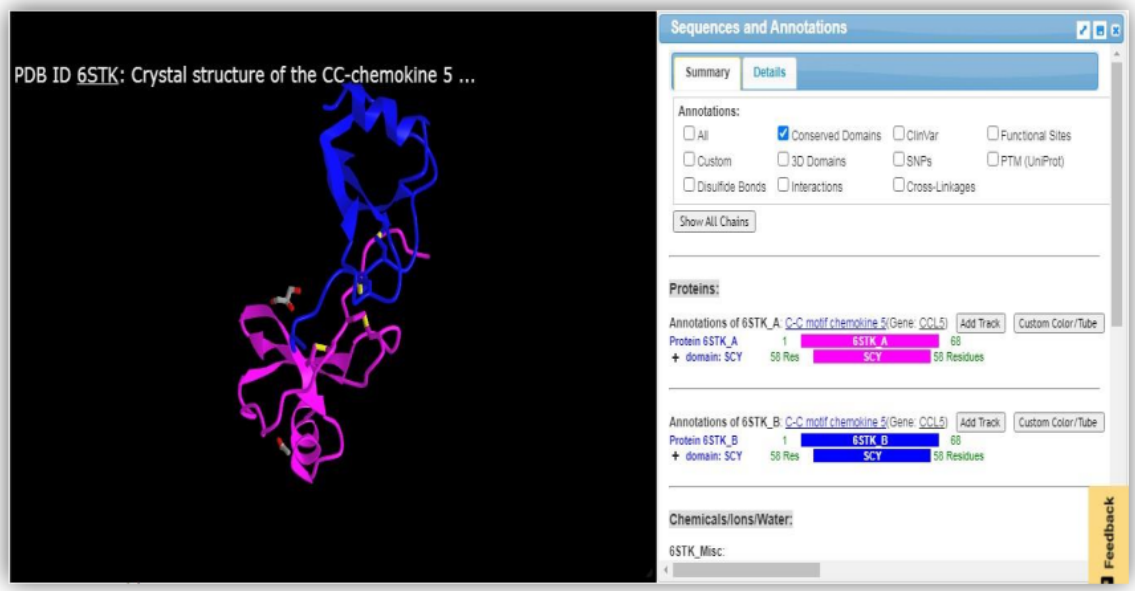
Score	Expect	Methods	Identity	Positive	Gap
153 bits(386)	3e-46	Compositional adjust.	matrix91/91(100%)	91/91(100%)	0/91(0%)

```
Query  1   MKVSaaalaviliatalcaPASASPYSSDTTPCCFAYIARPLPRAHIKEYFY
           TSGKCSNP  60
           MKVSAAALAVILIATALCAPASASPYSSDTTPCCFAYIARPLPRAH
Sbjct  1   IKEYFYTSGKCSNP
           MKVSAAALAVILIATALCAPASASPYSSDTTPCCFAYIARPLPRAH
           IKEYFYTSGKCSNP  60

Query  61  AVVFVTRKNRQVCANPEKKWVREYINSLEMS  91
           AVVFVTRKNRQVCANPEKKWVREYINSLEMS
Sbjct  61  AVVFVTRKNRQVCANPEKKWVREYINSLEMS  91
```

Figure 3. Showing the similarity and difference between the protein translated fromthe sequence query and by comparing it with the source protein, as the letters symbolize the types of amino acids.

https://www.ncbi.nlm.nih.gov/Structure/icn3d/full.html?&mmdbid=191888&bu=1&showanno=1&source=f



ull-feature

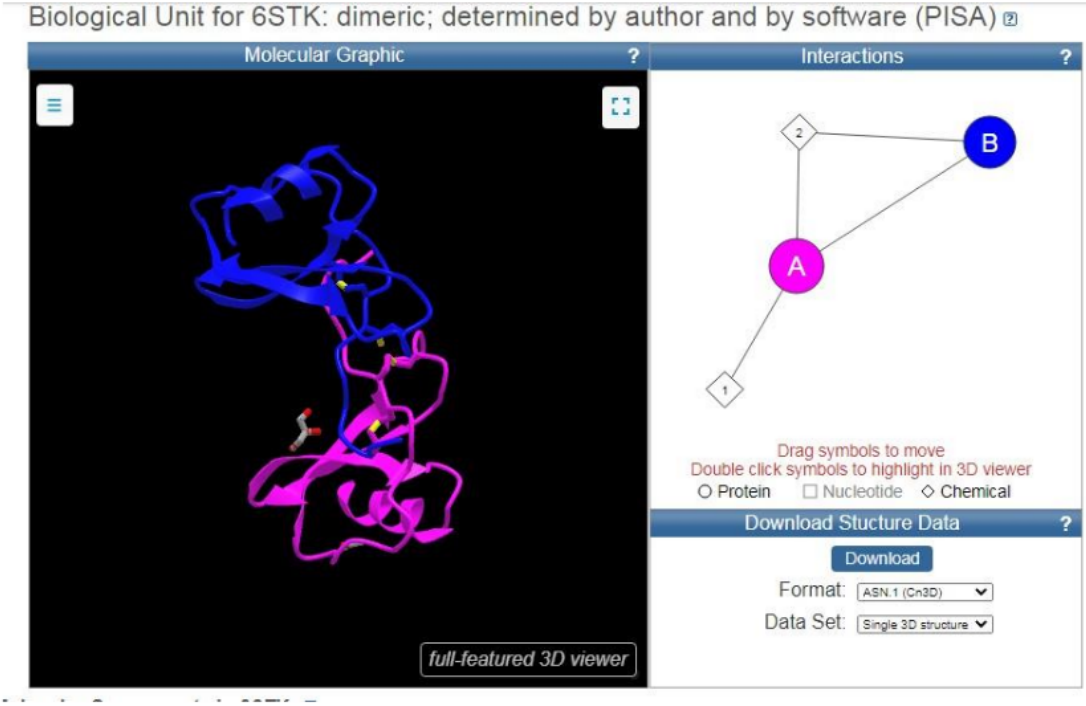


Figure 4. Protein structure of the CCL5.

3D Protein Structure for CXCL-10

C-X-C motif chemokine CXCL10precursor [Homo sapiens] NCBI-2024 Reference Sequence: NP_001556.2

GenPept Identical Proteins Graphics

>NP_001556.2 C-X-C motif chemokines 10 precursors [Homo sapiens]

MNQTAILICCLIFLTLSGIQGVPLSRTVRCTCISISNQPVNPRSLEKLEIIPASQFCPRVEIIATMKKK

GEKRCLNPESKAIKNLLKAVSKERSKRSP

C-X-C motif chemokine CXCL10 precursor [Homo sapiens] Sequence ID: NP_001556.2Length: 98Number of Matches: 1 Range 1: 56 to 83GenPeptGraphics

Alignment statistics for match #1						
Score	Expect	Methods	Identity	Positive	Gap	Frame

44.3 bits(103)	0.006	Compositional adjust.	matrix	22/28(79%)	22/28(78%)	0/28(0%) +1

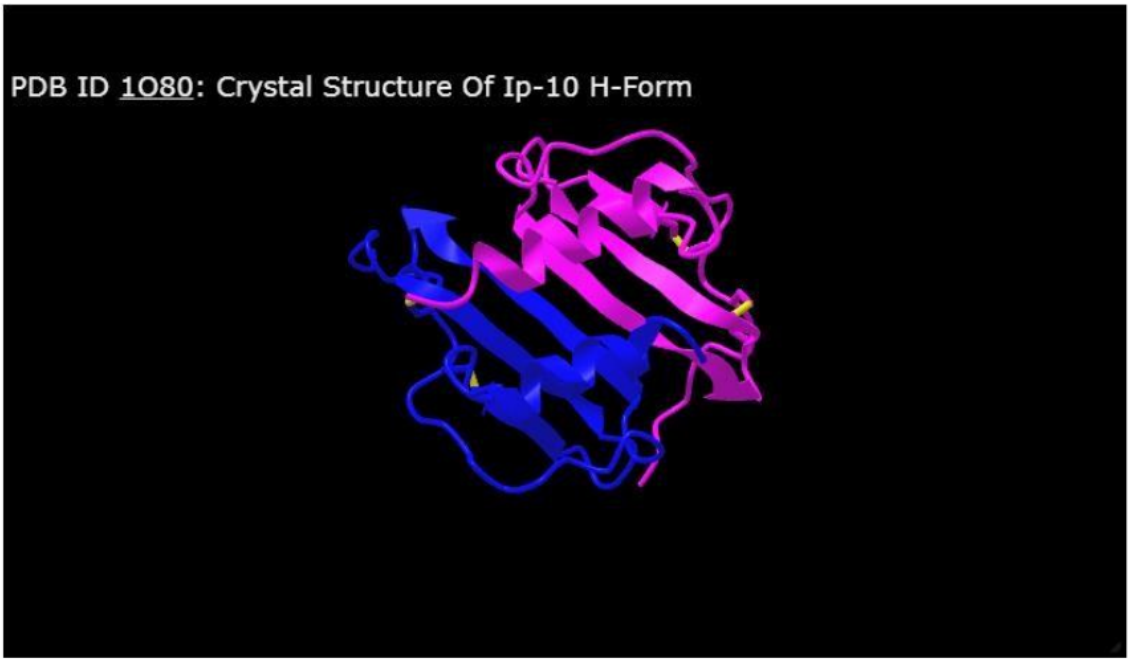
Query	217	FIPIYFYSATMKKKGEKRCLNPESKAI 300
		K
		F P
		ATMKKKGEKRCLNPESKAI
		K
Sbjct	56	FCPRVEIIATMKKKGEKRCLNPESKAI 83
		K

Query	217	FIPIYFYSATMKKKGEKRCLNPESKAI 300
		K
Sbjct	56	**.C.RVEII**..... 83

Figure 5. Showing the similarity and difference between the protein translated from the sequence query and by comparing it with the source protein, as the letters symbolize the types of amino acids.

C-X-C motif chemokines 10 precursors [Homo sapiens] Sequences ID: NP_001556.2Length: 98Number of Matches: 1

See 7 more title(s) See all Identical Proteins (IPG)



https://www.ncbi.nlm.nih.gov/Structure/icn3d/full.html?&mmdbid=23068&bu=1&showanno=1&source=full-feature

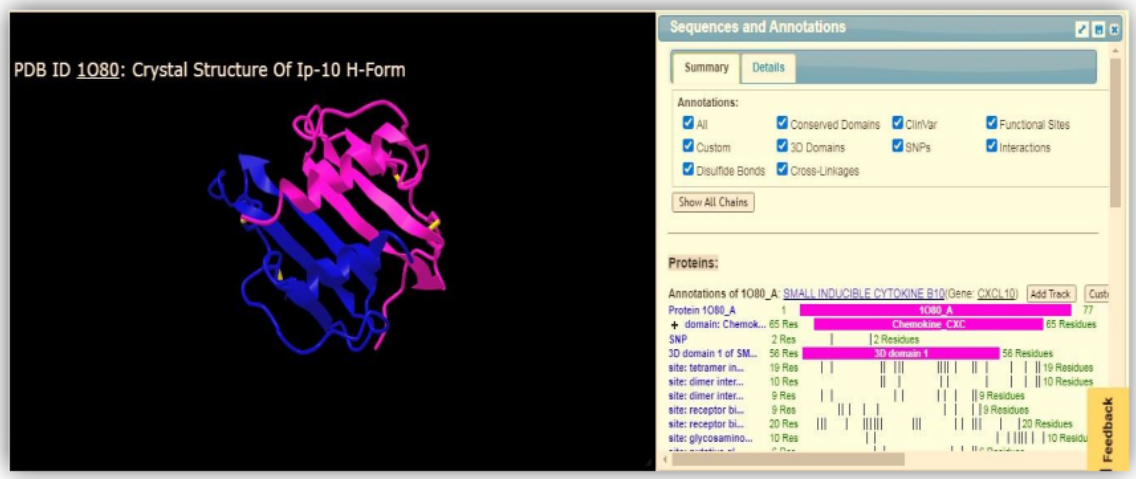


Figure 7. Crystal Structure of Ip-10 H-Form with CXCL10

Table 2. Showing wild-types and amino acids variation after DNA molecules exposure to SNP mutations at different locations.

Sample No.	Wild Types (Subjects)	Amino acid Variation (Query)
2	C (Cysteine)	I (Isoleucine)
	R (Arginine)	I (Isoleucine)
	V (Valine)	Y(Tyrosine)
	E (Glutamic acid)	F (Phenylalanine)
	I (Isoleucine)	Y(Tyrosine)
	I (Isoleucine)	S(Serine)

DISCUSSION

The present study sequencing of CCL-2, CCL-5 and CXCL-10 showed there a convergence between study CCL-2 isolated and that of the Gen-Bank databases (NCBI-2024) with identities 19/20 (95%) CCL2 had a one mutations appeared as (D to E). While there a high convergence between study CCL5 isolated and that of the GenBank databases (NCBI-2024) with identities 91/91 (100%). And there a low convergence between study CXCL10 isolated and that of the GenBank databases (NCBI-2024) with identities 22/28 (79%) in CXCL10 had a Six mutations appeared as (C to I), (R to I), (V to Y), (E to F), (I to Y), and (I to S).

No previous works interested studied in 3D-protein with chemokines, so we cannot discuss the current research.

No studies about chemokines of MS in Iraq, so the present study is found necessary as a first study to determine chemokines 3D protein structure among patients with MS in Iraq –generally- and in Basrah –especially.

CONCLUSION

There a convergence between study CCL-2 isolated CCL-2 had a one mutations appeared as D (Aspartic acid) to E (Glutamic acid). While there a great convergence between study CCL-5 isolated. And there a low convergence between study CXCL-10 isolated and CXCL10 had a six mutations seen as C (Cysteine) to I (Isoleucine), R (Arginine) to I (Isoleucine), V(Valine) to Y(Tyrosine), E (Glutamic acid) to F(Phenylalanine), I(Isoleucine) to Y(Tyrosine), and I(Isoleucine) to S(Serine).

Table 3. Amino acids and codons of the 3D protein structures.

Amino acids	Symbols		Codons
Alanine	Ala	A	GCA, GCC, GCG, GCU
Cysteine	Cys	C	UGC, UGU
Aspartic acid	Asp	D	GAC, GAU
Glutamic acid	Glu	E	GAA, GAG
Phenylalanine	Phe	F	UUC, UUU
Glycine	Gly	G	GGA, GGC, GGG, GGU
Histidine	His	H	CAC, CAU
Isoleucine	Ile	I	AUA, AUC, AUU
Lysine	Lys	K	AAA, AAG
Leucine	Leu	L	UUA, UUG, CUA, CUC, CUG, CUU
Methionine	Met	M	AUG
Asparagine	Asn	N	AAC, AAU
Proline	Pro	P	CCA, CCC, CCG, CCU
Glutamine	Gln	Q	CAA, CAG
Arginine	Arg	R	AGA, AGG, CGA, CGC, CGG, CGU
Serine	Ser	S	AGC, AGU, UCA, UCC, UCG, UCU
Threonine	Thr	T	ACA, ACC, ACG, ACU
Valine	Val	V	GUA, GUC, GUG, GUU
Tryptophan	Trp	W	UGG
Tyrosine	Tyr	Y	UAC , UAU

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