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New Approach for Vaccine or Immune Boosting by Immunoinformatics Survey to Detect Cross Reacted E. Coli Antigens with COVID-19 Antibodies

Conflict of interest: nothing to declare.
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Ethics statement. Based on the requirements of the division of graduate studies in the College of the Pharmacy / University of Basra, a research proposal explaining the purpose of the study and methods for data collection was submitted to the university committee. After approval, the proposal of the current study was submitted to the committee of the Basrah Health Directorate, to grant ethical approval. The committee of the mentioned center approved that.
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Abstract

Introduction. Vaccine development has been sped up to obtain immunity to the virus and put an end to transmission as many countries continue to fight off infections caused by COVID-19.
Purpose. To introduce a new approach to apply of immunoinformatic to detect sharing epitopes among normal flora and probiotics with COVID-19 spikes as an attempt to be as human immune stimulator or vaccine.
Materials and methods. The patients age range was (18–90 years) of either sex. The rapid SARS-CoV-2 IgG-IgM combined antibody test kits were tested at three hospitals, with a total of 80 clinical positive and 20 clinical negative patient blood samples. A cross-sectional observational study was carried out. By using National Center for Biotechnology Information (NCBI). To detect which bacteria and parts of bacteria are similar to COVID-19 Spike protein. A promising method for stopping viral infections is the use of a multi-epitope peptide (MEP) made from overlapping immunodominant epitopes. Several bacterial strains using to detect cross-reaction between this bacteria and IgG of a vaccinated patient. The predicted vaccine's 3D-structure was modeled using the online server RaptorX. Then modeled structure was refined using the online server for the Galaxy refine tool, so used RAMPAGE to validate the improved 3-D structure. Namely, E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacteria and Gammaproteobacteria.
Results. It has been found that NCBI protein BLAST the bacteria E. coli outer site moiety which have more similarity percentage (42.43%) in relation to spike COVID-19. Immunoinformatic has been used to identify potentially immunogenic T cell epitopes of SARS-CoV-2 that could be used in a multi-epitope vaccine against the most widely distributed and potentially dangerous SARS-CoV-2 variants (VOC). These are hypothesized to be safe for human consumption, contain no allergens, and stimulate the immune

system. By NCBI can Vaccination strategies developed to elicit an immune response directed at these conserved epitopes have the potential to produce immunity that is not only protective against multiple strains of Beta coronavirus but also resistant to the virus's ability to adapt as mention mean proteins of COVID-19.

Conclusion. A promising method for stopping viral infections is the use of a multi-epitope peptide (MEP) made from overlapping immunodominant epitopes. The predicted vaccine's 3D-structure was modeled using the online server RaptorX. Then modeled structure was refined using the online server for the Galaxy refine tool, so used RAMPAGE to validate the improved 3D-structure.

Keywords: SARS-CoV-2, COVID-19, multi-epitope peptide, Pseudomonas aeruginosa, Klebsiella pneumoniae

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Новый подход к применению вакцины или иммунного бустера на основании результатов иммуноинформационного анализа по выявлению перекрестных реакций антигенов E. coli с антителами к COVID-19

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Этическое заявление. На основании требований отдела аспирантуры Фармацевтического колледжа Университета Басры в университетский комитет было подано ходатайство о проведении исследования, объясняющее его цель и методы сбора данных. После одобрения данное предложение было направлено в комитет Управления здравоохранения Басры, который также принял положительное решение.
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Резюме

Введение. Необходимость формирования иммунитета к вирусу COVID-19 и недопущение его распространения стали стимулом для ускоренной разработки вакцин, поскольку многие страны по-прежнему вынуждены противостоять инфекциям, вызванным этим вирусом.

Цель. Представить новый подход к применению иммуноинформационного метода для выявления общих эпитопов нормальной микрофлоры и пробиотиков со спайковым белком COVID-19 в целях возможного использования их в составе иммуностимуляторов или вакцин для человека.

Материалы и методы. В исследовании участвовали пациенты обоих полов в возрасте от 18 до 90 лет. Экспресс-тесты на антитела IgG-IgM к вирусу SARS-CoV-2 были выполнены в трех больницах; всего было исследовано 100 пациентов (60 мужчин, 40 женщин). Анализу подлежали 80 клинически положительных и 20 клинически отрицательных образцов крови пациентов. Было проведено перекрестное обсервационное исследование с использованием данных Национального центра биотехнологической информации (NCBI) для выявления бактерий и отдельных участков бактерий, схожих со спайковым белком COVID-19. Перспективным методом подавления вирусных инфекций является использование мультиэпитопного пептида (MEP), состоящего из перекрывающихся иммунодоминантных эпитопов. Для выявления перекрестной реакции между бактериями и IgG вакцинированного пациента использовали штаммы нескольких бактерий. Предполагаемую 3D-структуру вакцины смоделировали с помощью онлайн-сервера RaptorX. В дальнейшем смоделированная структура была усовершенствована с помощью инструментов онлайн-сервера Galaxy, после чего с помощью RAMPAGE улучшенная 3D-модель была верифицирована. В качестве объектов исследования были выбраны E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacteria и Gammaproteobacteria.
Результаты. Установлено, что белок внешней мембраны молекулы бактерии E. coli, выявленный с помощью алгоритма BLAST на основании данных NCBI, имеет наибольший процент сходства (42,43%) со спайковым белком COVID-19. Для выявления потенциально иммуногенных Т-клеточных эпитопов SARS-CoV-2, которые, предположительно, могут быть использованы в мультиэпитопной вакцине против наиболее широко распространенных и потенциально опасных вариантов SARS-CoV-2 (VOC), был применен иммуноинформационный метод. Предполагается, что исследуемые биологические объекты безопасны для человека, не содержат аллергенов и стимулируют иммунную систему. Базирующаяся на основании данных Национального центра биотехнологической информации (NCBI) разработка стратегий вакцинации с целью вызвать иммунный ответ, направленный на данные консервативные эпитопы, позволит сформировать иммунитет, обеспечивающий защиту от различных штаммов коронавируса Beta и устойчивый к адаптационным свойствам вируса, например, к белкам COVID-19.
Заключение. Перспективным методом подавления вирусных инфекций является использование мультиэпитопного пептида (MEP), состоящего из перекрывающихся иммунодоминантных эпитопов. Предполагаемую 3D-структуру вакцины смоделировали с помощью онлайн-сервера RaptorX. В дальнейшем смоделированная структура была усовершенствована с помощью инструментов онлайн-сервера Galaxy, после чего с помощью RAMPAGE улучшенная 3D-модель была верифицирована.
Ключевые слова: SARS-CoV-2, COVID-19, мультиэпитопный пептид, Pseudomonas aeruginosa, Klebsiella pneumoniae

■ INTRODUCTION

Coronavirus was globally first encountered in 2002–2003 as a result of Severe Acute Respiratory Syndrome (SARS), then in 2011 as a result of Middle East Respiratory Syndrome

(MERS), both instances were caused by newly found coronaviruses of zoonotic origin belonging to the genus Beta-coronavirus [1].

The molecular structure of SARS-CoV-2 has four key proteins: the spike (S), envelope (E), membrane (M), and nucleocapsid (N). SARS-CoV-2 shares approximately 80% of its genetic sequence with SARS-CoV the virus causative for the pandemic of SARS in 2002. Despite the similarities, the Severe acute respiratory S protein which enables the virus to connect to the angiotensin-converting enzyme ACE2 receptor is many amino acids lengthier than the SARS-CoV S protein [2].

Vaccine development has been sped up to obtain immunity to the virus and put an end to transmission as many countries continue to fight off infections caused by COVID-19. Usually, making a vaccine takes a long time and is a lot of work. Recent progress in making a vaccine against COVID-19 has shown that research advances build on what is already known and add to it [3, 4].

Improvements to the alignment display were first included in the Power BLAST client and then incorporated into the BLAST Graphical Overview section of the NCBI BLAST report. Many usability difficulties with the BLAST reports on the NCBI website remained, despite these updates [5]. By NCBI can Vaccination strategies developed to elicit an immune response directed at these conserved epitopes have the potential to produce immunity that is not only protective against multiple strains of Beta coronavirus but also resistant to the virus's ability to adapt as mention mean proteins of COVID-19 [6, 7].

Screening of bacterial antigens that have cross-reactivity with the COVID-19 antibodies, which may have a role in immune enhancement by following steps: Using to compare and detect possible similarities between COVID-19 spike and several bacteria by 3-dimensional protein structure for COVID-19 spike and bacteria.

■ PURPOSE OF THE STUDY

To introduce a new approach to apply of immunoinformatic to detect sharing epitopes among normal flora and probiotics with COVID-19 spikes as an attempt to be as human immune stimulator or vaccine.

■ MATERIALS AND METHODS

Study design and settings

Between December 2021 and April 2022, a cross-sectional observational study was carried out, the study was conducted at (Basrah teaching hospital / Basrah governorate / Iraq). The ethical and scientific committee of the faculty of pharmacy / Basra university in addition to the scientific committee of research of a Basra health directorate approved this study.

Patient's selection

Only one hundred patients, N=100 (60 males and 40 females) were included in the study, who agreed with the study.

All samples were taken from healthy persons that only vaccinated with the Pfizer vaccine about 80 and non-vaccinated about 20 persons. Generally, the inclusion criteria are: The ranged age of patients was (18-90 years) of either sex, patients and relatives should be able to communicate and be willing to participate in this study and patients who were vaccinated with Pfizer company only. While the exclusion criteria are: patients

with acute infectious diseases like pneumonia, and human immunodeficiency virus in addition to patients who had been vaccinated with any type of vaccine except Pfizer.

Sample collection

After 2 weeks of a vaccinated patient by Pfizer company with healthy in origin with age generally above 18 years old. Also taken from a non-vaccinated healthy human with an age above 18 years.

Blood sample

Participants had their veins punctured in the usual way, and put in red-topped SST tubes 5 ml of whole blood was collected by gel tube (Medise). Once the sample was collected, it was allowed to coagulate at room temperature for 30–60 minutes before being centrifuged at 2,500 rpm for 15 minutes. Specimens were stored on ice for no more than 6 hours before being transported to the laboratory, where the serum was aliquoted to the appropriate volumes and frozen at –20 °C until use [8].

Serum tested

After the serum is taken from the vaccinated patient must be tested to detect the presence of IgG or IgM, that done according to manufacturing data.

It was unzipped and ready to use right before testing began. Blood samples stored in the refrigerator were brought to room temperature (15–30 °C) before being used in the analysis. Two to three drops (70–100 µL) of dilution buffer (10 mM PBS buffer) were added to the sample port to drive capillary action along the strip after a 20 µL whole blood sample (or 10 µL of serum/plasma samples) was pipetted in and about 15 minutes were needed to complete the entire exam [9].

NCBI

By using National Center For Biotechnology Information (NCBI) [10]. To detect which bacteria and parts of bacteria are similar to COVID-19 Spike protein. when open this site of internet search will be done on the nucleotide of a spike. Generally, the sequences of the COVID-19 Spike protein (Surface glycoprotein) are mentioned below [11]. Also, NCBI can get the sequence of the all bacteria used in the study.

Selection priorities for COVID-19 vaccines

The full catalog of coronavirus proteins is now accessible through the (NCBI) protein data hub [10]. Was retrieved, and the screening phase began to determine which ones would make good vaccines. The pathogen's proteins with no human-host homology were first filtered out (taxonomic id: 9606), Proteins were chosen with a sequence identity of less than 30%, a bit score greater than 100, and a sequence E score of less than $1.0E^{-5}$ [12]. Next, we used the BLASTp tool to align the selected adhesive protein candidates with some types of bacteria like E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacteria and Gammaproteobacteria [13]. Also, the probiotic bacteria proteome, which includes: Lactobacillus species, Bifidobacterium species, Streptococcus thermophilus, Saccharomyces boulardii, Bacillus clausii and Bacillus subtilis to prevent unintentional suppression of the beneficial gut bacteria [14].

Epitope mapping of B- and T-cells

The immune epitope database was subsequently applied to the chosen vaccine candidates (IEDB) The 2.0 version of the Bepipred Linear Epitope Prediction algorithm [15]. The threshold of 0.5 was used to predict linear B-cell epitopes, which were then used in T-cell epitopes mapping to find subsequences that could bind to the reference set of major histocompatibility complex (MHC) class I and II alleles [16]. Epitopes were ranked by their percentile score, and the ones with a low percentile were considered to have a high affinity for binding, the selected B-cell-derived T-cell epitopes were then run through MHCPred 2.0 [17].

Multi-epitope peptide (MEP) synthesis and adjuvating

A promising method for stopping viral infections is the use of a multi-epitope peptide (MEP) made from overlapping immunodominant epitopes [18]. Weak immunogenicity is a major problem in peptide vaccine design, but this can be fixed by creating an MEP with the right adjuvants [19]. Bioinformatics methods are used to predict B- and T-cell epitopes from the novel S protein of SARS-CoV-2, paving the way for the creation of a novel multiple epitope vaccine, to determine which epitopes would be most effective at eliciting an immune response, we considered their immunogenicity, antigenicity scores, and toxicity. The most promising multi-epitope in terms of its immunogenic potential was built using epitopes. Linkers of the types EAAAK, AAY, and GPGPG were used to bind the epitopes together [20]. By performing a backwards translation of the MEPVC sequence and then optimizing it for codon usage in accordance with Escherichia coli, we were able to increase the expression of the MEPVC sequence cloned in the aforementioned expression system [21].

Analysis physicochemical properties of MEPVC

In order to better inform experimental investigations, the physical and chemical properties of MEPVC were analyzed using the ProtParam tool, these properties included the amino acid composition, estimated half-life, instability index, extinction coefficient, theoretical pl atomic composition, molecular weight, and grand average of hydropathicity (GRAVY). The instability index is one of the most important factors to take into account because it allows one to eliminate protein candidates that are too unstable (i.e., have an instability index greater than 40) [22]. C-ImmSim used a position-specific scoring matrix (PSSM) to make predictions about immune epitopes.

However, the immune connections were predicted using a variety of machine learning procedures. Three immune system compartments, including bone marrow, tertiary lymph nodes, and the thymus, are simulated on this server at once. The host HLA was chosen as follows: A MHC class I A0101 allele, B MHC class I B0702, DR MHC class II DRB1 0101 allele, and the time step of injection was set to 1, the default random seed was 12345, and the simulation steps were 100 and the simulation volume was 10 [23].

MEPVC docking process

Molecular docking was used to determine which innate immune receptor would be the best fit for predicting the MEPVC's conformation, this analysis is crucial for identifying the vaccine construct's high-affinity contacts with the immune receptor [24]. In this study used docking between spike glycoprotein of COVID-19 and Various types

of bacteria (E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacteria and Gammaproteobacteria) so the typical orientation of the vaccine construct with the aforementioned receptors was calculated using blind docking [25].

3D-structure prediction

The predicted vaccine's 3D-structure was modeled using the online server RaptorX [26]. Then modeled structure was refined using the online server for the Galaxy refine tool, so used RAMPAGE to validate the improved 3D-structure [27–29].

Bacterial Strains

Several bacterial strains using to detect cross-reaction between this bacteria and IgG of a vaccinated patient as shown in table 1.

Table 1
Bacterial isolate and supplier origin

No	Strain of Bacteria	Supplier
1	Staphylococcus aureus	University of Basrah / college of pharmacy
2	Pseudomonas aeruginosa	University of Basrah / college of pharmacy
3	Escherichia coli	Al-sadder teaching hospital
4	Klebsiella pneumoniae	University of Basrah / college of pharmacy
5	Bacillus	Al-sadder teaching hospital
6	Enterobacteria	Al-sadder teaching hospital

■ RESULTS

NCBI and immunoinformatic

The BLAST search was done using web portal surface glycoprotein of COVID-19 Protein in NCBI server and other bacterial sequences were done using web portal Protein BLAST: search protein databases using a protein query by NCBI web site was used to all bacterial protein used in the study. BLAST found regions of homology between the protein sequences of COVID-19 spike glycoprotein with those pathogenic bacteria. By Clustal Omega server the bacterial genome sequences are found in both pathogenic and commensal bacteria that are known to be infectious in related to Genene sequences of spike COVID-19. Namely, E. coli, Pseudomonas aeruginosa, klebsiella pneumonia, Enterobacteria and Gammaproteobacteria.

From table 2 by NCBI, then choice BLAST the bacteria protein of E. coli outer site moiety which have more similarity percentage (42.43%) in relation to spike COVID-19. While found the other bacteria like Pseudomonas aeruginosa outer site moiety similarity percentage (21.00%) in relation to spike COVID-19. But, also find outer site moiety of Klebsiella pneumonia, Enterobacteria and Gammaproteobacteria similarity percentages about (16.22%) in relation to spike COVID-19. While the probiotics like Lactobacillus species, Bifidobacterium species, Streptococcus thermophilus, Saccharomyces boulardii and Bacillus Species not find any NCBI matching.

Escherichia coli epitope and similarity with COVID-19

Alignment of Escherichia coli

The alignment of the COVID-19 spike protein with the E. coli protein show in fig. 1. Both of these proteins shared 42.43% identity percentage of similarity represented in

Table 2
Description the genome similarity percentage among COVID-19 spike glycoprotein and some bacterial isolates

No	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1	Coronavirus S2 glycofamilyprotein [Escherichia coli 1-110-08_S3_C3]	Escherichia coli 1-110-08_S3_C3	913	913	46%	0.0	42.43%	570	EYE09374.1 Below threshold ofprevious iteration
2	PAS domain-containing protein [Pseudomonas aeruginosa].	Pseudomonasaeruginosa	249	249	16%	6e-69	21.00%	521	MBG4596959.1 below threshold ofprevious iteration
3	methyl-accepting chemotaxis protein [Klebsiella pneumoniae]	Klebsiella pneumoniae	92.1	92.1	12%	2e-17	16.11%	283	WP_181964373. below threshold ofprevious iteration
4	methyl-accepting chemotaxis protein [Enterobacteria]	Enterobacteria	91.7	91.7	12%	2e-17	16.11%	265	WP_211732038. below threshold ofprevious iteration
5	PAS domain-containing methyl-accepting chemotaxis Protein [Gammaproteobacteria]	Gammaproteobacteria	92.5	92.5	12%	2e-16	16.11%	536	WP_234262184. Below threshold ofprevious iteration

some motifs in table 2. The Similarity began from ($TNFT^{715\text{-}719}$) of COVID-19 spike peptide sequence and E. coli from ($TNFS^{1\text{-}4}$) peptide sequence, that each black background of a. a means Sometimes they are 100% identical when compared to alignment and sometimes there is a difference in one or two letters between the virus and bacteria, we focused on the top match. Sometimes you find the letters different, but in alignment they take the same black color and indicate that they are not different, and the reason is due to these security acids similar in structure as well as function. That's why it's considered identical. More

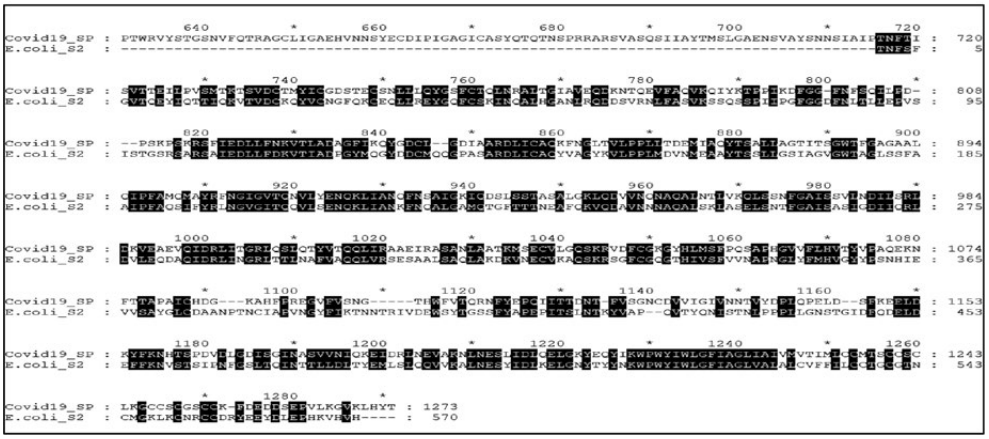

Fig. 1. Alignment of similarity relationship between COVID-19 spike protein and E. coli protein

similarity of COVID-19 spikes peptide (IEDLLFNKVTLAD[821-834], KWPWYIWLGFIAGLIAI[1228-1244]) and E. coli Peptide (IEDLLFNKVTLAD[107-119], KWPWYIWLGFIAGLVAI[520-527]). So we can cut these sequences and by multiple process can be developed and synthesis new vaccine against COVID-19.

Secondary and tertiary structure evaluation

MEV Secondary structure was analyzed with help of server RaptorX property, while The MEV's predicted 3D structure was created in 3Dpro. In this case, we used the de novo approach because we didn't have access to a suitable PDB template. As a result, the Galaxy Refine server was used to refine the predicted structure and increase its quality, the five resulting models were more accurate as a result of the structure being improved by this server. Ramachandran plot, ProSA, and ERRAT score were also used to further assess these models. According to these findings, the optimal model was chosen for additional testing.

From below fig. 2 in Both A, B and C the result of docking between E. coli with COVID-19 Spike where the red color represents Spike COVID-19 Protein that docking by various types of bonding with green Color represent E. coli Protein.

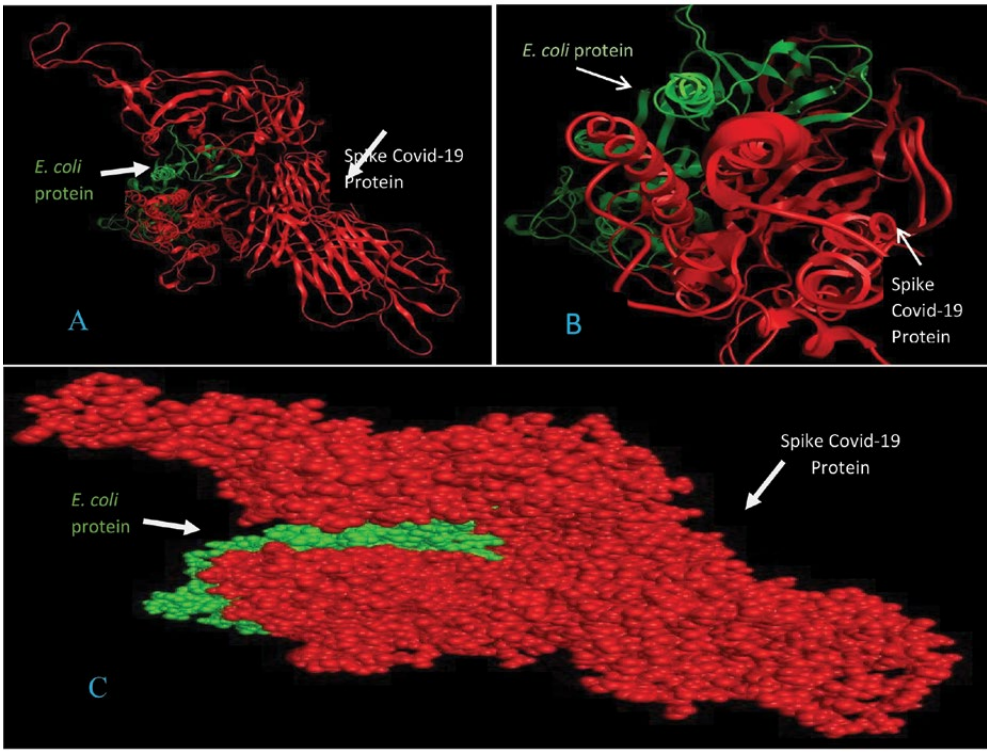

Fig. 2. The 2D- Structure of E. coli docking with COVID-19 Spike for Both A, B and 3D-structure of C that represent (red color to Spike COVID-19 Protein while green Color to E. coli Protein)

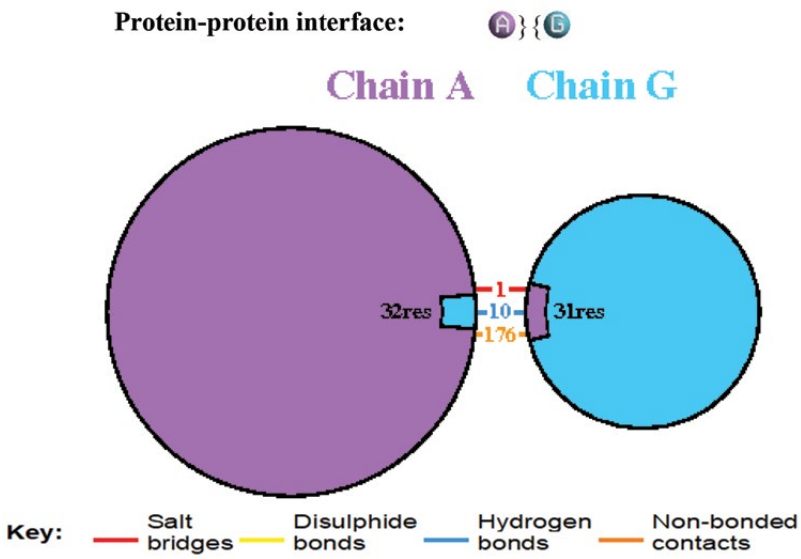

Fig. 3. Protein-Protein interference between Spike COVID-19 (chain A) and E. coli (chain G)

Schematic depiction of how protein-chain interactions work. Varying types of interactions are indicated by different colors of connecting lines between chains. The surface area of each protein chain is shown by the size of its matching circle. The black wedge's size represents the interface surface area, which in turn represents the extent of the interface zone on each chain. Statistics for this interface between spike COVID-19 and E. coli represent number of salt bridges is 1, and number of H-bonding are 10 while number of unbonded contacts is 176 as mention in table 3.

Residue interactions across interface Colored by residue type. To evaluate the interaction between Spike COVID-19 glycoprotein, and E. coli, The ClusPro 2.0 server was used for blind molecule docking. The best docked complex was determined by looking for the docking models with the largest cluster sizes and lowest interaction energy scores. This suggests that the complexes have adequate binding affinity and occupies the receptor in the right way. After the best-docked complexes were chosen, PDBsum was used to display the interactions at the molecular level between the complex and its receptors. Predicted for each complex were receptor and MEV interface area and also number of interacting

Table 3
Interfacial statistics between spike COVID-19 protein and E. coli protein

No	Chain	No. of interface residues	Interface area (A2)	No. of Salt bridges	No. of Disulphide bonds	No. of hydrogen bonds	No. of non-bonded contacts
1	A	32	1647	1	—	10	176
2	G	31	1725				

Interaction of Spike COVID-19 with potential receptors of E. coli

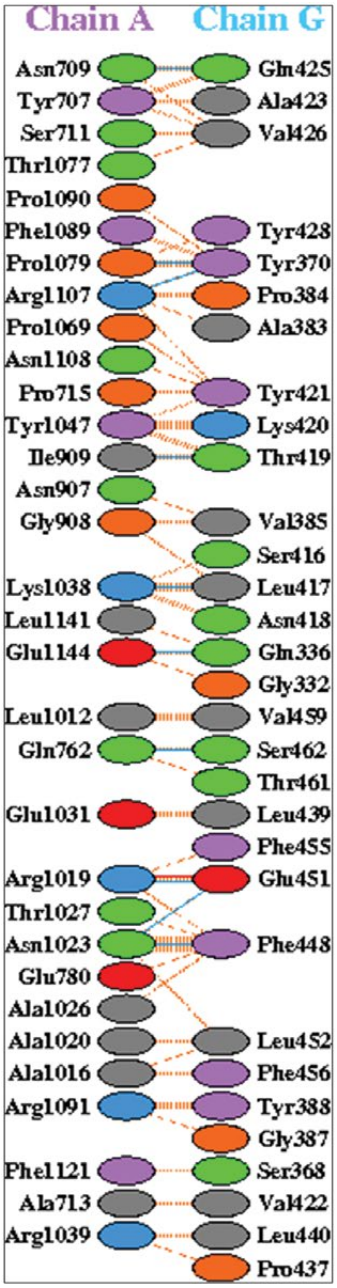

Fig. 4. Docked complexes between the spike COVID-19 (Chain A) and E. coli (Chain G)

Bond Key: Salts-bridge Di-sulphide bond Hydrogen bond non-bonded contact.
Colors for Residues are as follows: Positively (R, K, H); negatively (E, D); neutrally (Q, T, N, S); aromatic (W, Y, F); aliphatic (M, I, L, V, A); cysteine (C) and Gly. And Pro. (G, P).

interface residues. It was also possible to make educated guesses about how many salt-bridges, hydrogen-bonds, and non-bonded contacts would form between the two of them. The findings revealed that the MEV established numerous interactions with each receptor. Each line of H-bond that bind any pairs of a.a indicates the potential number of H-bonds between these pairs. while unbonded contacts that may be plenteous striped line width that directly proportional atomic contacts' number as mention in fig. 4.

Hydrogen bond analysis

Hydrogen bonding takes place when an electronegative atom attracts a hydrogen atom that is already bound to it and play important role in determining specificity and directionality of molecular recognition in a biological system. To investigate the stability of the intermolecular association over the course of the simulation, the hydrogen-bonding patterns of both complexes were depicted in each frame within 3 A°. Spike of COVID-19 forms a maximum of 10 hydrogen bonds with E. coli protein, demonstrating the potency of these interactions. Table 4 displays the total number of hydrogen bonds present in both complexes.

Table 4
Hydrogen bonded contacts between spike COVID-19 protein and E. coli protein

Spike COVID-19 Protein						E. coli Protein					
No.	**Name of Atom**	**No. of Atom**	**Name of Res**	**Number of Res**	**The Chain**	**Name of Atom**	**No. of Atom**	**Name of Res**	**No. of Res**	**The Chain**	**The Distance**
1.	6709	ND2	ASN	709	A	14759	O	GLN	425	G	2.96
2.	7187	NE2	GLN	762	A	15105	OG	SER	462	G	2.86
3.	8561	O	ILE	909	A	14697	OG1	THR	419	G	2.77
4.	9608	NE	ARG	1019	A	14996	OE1	GLU	451	G	3.04
5.	9645	ND2	ASN	1023	A	14968	O	PHE	448	G	2.82
6.	9645	ND2	ASN	1023	A	14996	OE1	GLU	451	G	2.97
7.	9776	NZ	LYS	1038	A	14681	O	LEU	417	G	2.92
8.	10169	O	PRO	1079	A	14245	OH	TYR	370	G	2.71
9.	10435	NE	ARG	1107	A	14247	O	TYR	370	G	2.86
10.	10787	OE1	GLU	1144	A	13927	NE2	GLN	336	G	3.20

Non-bonded interactions

Particularly at the spike of COVID-19 protein and E. coli protein, replacing residues of polar acidic as glutamate with alternative polar acidic as asparagine, or any polar residues that have positively charged same to: arginine, lysine and histidine, by other charged residues of positively polar, has improved their ability to interact non-covalently with nearby residues and to residues in their corresponding receptor proteins. The striped line width indicates the density of unbonded contacts between atoms, that is generally high bounded. Total number in unbonded interaction between spike COVID-19 and E. coli are 176.

Like TYR^{707} of spike COVID-19 have non bonded contact with ALA^{423}, GLN^{525} were estimated in 3.84 ^{0}A and 3.86 ^{0}A respectively.

Salt bridges analysis

At neutral pH, salt bridges are formed in the protein molecule between the charged side chains of amino acids. Glutamine in E. coli, which has a negative full electron charge, and arginine in spike COVID-19 which has a positive full electron charge are two of the most important residues in these interactions [30]. A strong indication of increased interaction stability is the presence of salt bridges between the interacting molecules. For Spike COVID-19 – E. coli complex of salt bridges were estimated within 2.7 Å between receptor ARG^{1019} with E. coli GLU^{451} as mention in below table 5.

Table 5
Salt Bridges between spike COVID-19 protein and E. coli protein

Spike COVID-19 protein						E. coli protein					
No.	**Name of Atom**	**No. of Atom**	**Name of Res**	**Number of Res**	**The Chain**	**Name of Atom**	**No. of Atom**	**Name of Res**	**No. of Res**	**The Chain**	**The Distance**
1.	9611	NH2	ARG	1019	A	14997	OE2	GLU	451	G	2.70

Analysis of Epitope Mapping of B and T Cells

T Cell epitopes predication with MHCI and MHCII

T Cell epitopes predication with MHCI

Similarity between the peptide sequences of COVID-19 and those of pathogenic bacteria with identity ranging from 16.11% to 42.43% (table 2). The homology regions showed up to 0% difference, and 100% identity when they were processed as 9mers, the minimum length required for MHCI presentation to CD8+ T cells (CD8 functions as Tc (cytotoxic T) cells which recognize peptides displayed by MHC class I molecules) that between COVID-19 Protein (YP_009724390.1) Homology with E. coli as mention in table 6, while find 3-dimensional structures of part COVID-19 and E. coli shows the T cell epitope predication as mention in fig. 7.

Table 6
T Cell epitope predication with major histocompatibility complex 1 (MHC1) between spike COVID-19 peptide and E. coli peptide

No.	Organism	Peptide	Length	Difference (%)	Identity (%)
1.	COVID-19	IWLGFIAGL	9	0	100
	E. coli	IWLGFIAGL	9	0	100
2.	COVID-19	WPWYIWLGF	9	0	100
	E. coli	WPWYIWLGF	9	0	100
3.	COVID-19	RSFIEDLLF	9	11.1	88.9
	E. coli	RSAIEDLLF	9	11.1	88.9

The analysis of COVID-19 with MHCI as mention below peptide sequences, in this study epitope due to it net charge is 0, noted the charge and beta-sheet types not have positive and negative peptide so that appear in black color. While find in general formula pink color of peptide and amino acid (IWL, FIA, L) as hydrophobic properties but in Alpha-helix find (L, A) Amino acid have good interaction site but a.a (G) bad interaction site of COVID-19 with E. coli. Hydropathy plot show that most of the amino acid in vaccine are in favorable region.

Length: 9
Charge: IWLGFIAGL — red = positive, blue = negative
General: IWLGFIAGL — pink = hydrophobic, purple = P or G
Alpha-helix: IWLGFLAGL — green = good, orange = bad
Beta-sheet: IWLGFIAGL — green = good
M W: 989.2
Net charge: 0 (avg.: 0)
Hydropathy: 15.72 (avg.: 1.747)
Hydropathy Plot: as mention in fig. 5.

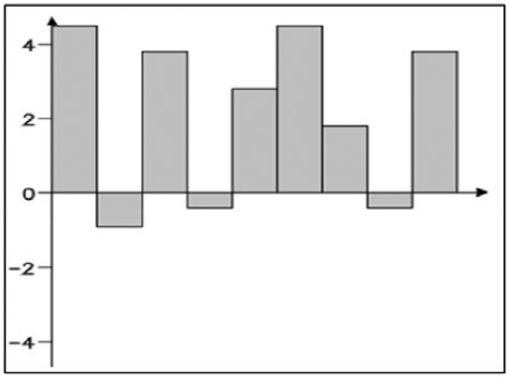

Fig. 5. Hydropathy plot of spike COVID-19 peptide (IWLGFIAGL)

The analysis of E. coli peptide with MHCI mention below peptide sequences, in this study epitope due to it net charge is 0, noted the charge and beta-sheet types not have positive and negative peptide so that appear in black color. While find in general formula pink color of a.a (W, I, A, L, F) as hydrophobic properties but in Alpha-helix find (L) a.a have good interaction site but a.a (G, Y, P) bad interaction site of E. coli with COVID-19. Hydropathy plot show that some of the amino acid in vaccine are in favorable region.

Length: 9
Charge: WPWYIWLGF — red = positive, blue = negative
General: WPWYIWLGF — pink = hydrophobic, purple = P or G
Alpha-helix: WPWYIWLGF — green = good, orange = bad
Beta-sheet: WPWYIWLGF — green = good
M.W: 1267.5
Net charge: 0 (avg.: 0)
Hydropathy: 3.21 (avg.: 0.357)
Hydropathy Plot: As mention in fig. 6.

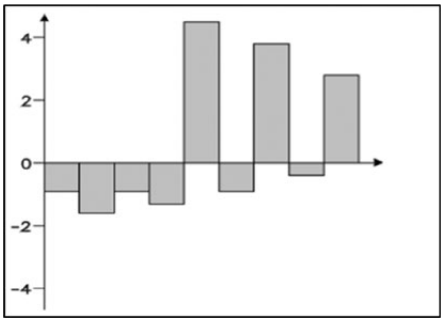

Fig. 6. Hydropathy plot of E. coli peptide (WPWYIWLGF)

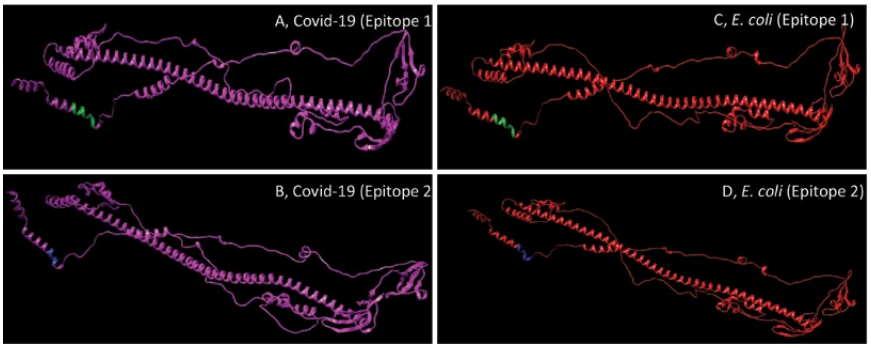

Fig. 7. A part of each 3-dimentianl structure of COVID-19 and E. coli shows the T cell epitope predication. The green color represents the peptide IWLGFIAGL in both COVID-19 and E. coli whereas the blue color represents the peptide WPWYIWLGF in COVID-19 and E. coli

T cell epitopes predication with MHCII

While MHCII (CD4 T cells function as T helper (Th) cells that recognize peptides displayed by MHC class II molecules) Homology between 15mers peptide of Spike COVID-19 Protein (YP_009724390.1) with E. coli, that represent difference Percentage as 6.7%, 20% and 26.7% with identified percentage 93.3%, 80% and 73.3% respectively as mention in table 7, while find 3-dimensional structures of part COVID-19 and E. coli shows the T cell epitope predication as mention in fig. 10.

Table 7
T cell epitope predication with major histocompatibility complex 2 (MHC2) between Spike COVID-19 and E. coli

No.	Organism	Peptide	Length	Difference	Difference (%)	Identity (%)
1.	COVID-19	NQKLIANQFNSAIGK	15	4	26.7	73.3
	E. coli	NQKLIANKFNQALGA	15	4	26.7	73.3
2.	COVID-19	RSFIEDLLFNKVTLA	15	3	20	80
	E. coli	RSAIEDLLFDKVTIA	15	3	20	80
3.	COVID-19	WPWYIWLGFIAGLIA	15	1	6.7	93.3
	E. coli	WPWYIWLGFIAGLVA	15	1	6.7	93.3

The analysis of COVID-19 peptide with MHCII mention below peptide sequences, in this study epitope due to it net charge is 0, noted the charge and beta-sheet types not have positive and negative peptide so that appear in black color. While find in general formula pink color of peptide (W, IWL, FIA, LIA) as hydrophobic but a.a (P, G) have hydrophilic properties. In Alpha-helix find (L, A) peptide have good interaction site but a.a (P, Y, G) bad interaction site of COVID-19 with E. coli. Hydropathy plot show that most of the amino acid in vaccine are in favorable region.

Length: 15
Charge: WPWYIWLGFIAGLIA — red = positive, blue = negative
General: WPWYIWLGFIAGLIA — pink = hydrophobic, purple = P or G
Alpha-helix: WPWYIWLGFIAGLIA — green = good, orange = bad
Beta-sheet: WPWYIWLGFIAGLIA — green = good
M.W: 1806.2
Net charge: 0 (avg.: 0)
Hydropathy: 16.15 (avg.: 1.077)
Hydropathy Plot: As mention in fig. 8.

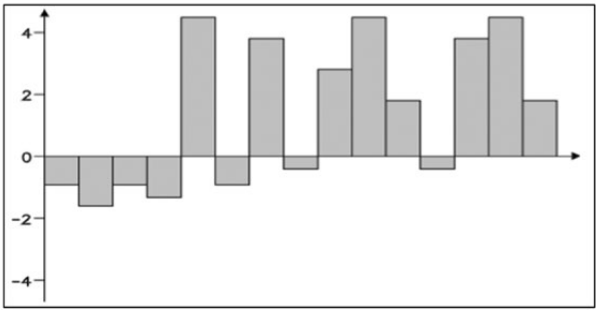

Fig. 8. Hydropathy plot of spike COVID-19 peptide (WPWYIWLGFIAGLIA)

The analysis of E. coli peptide with MHCII mention below peptide sequences, in this study epitope due to it net charge is 0, noted the charge and beta-sheet types not have positive and negative peptide so that appear in black color. While find in general formula pink color of peptide (W, IWL, FIA, LVA) as hydrophobic properties. In Alpha-helix find a.a (L, A) have good interaction site but a.a (P, Y, G) bad interaction site of E. coli with COVID-19. Hydropathy plot show that most of the amino acid in vaccine are in favorable region.

Length: 15
Charge: WPWYIWLGFIAGLVA — red = positive blue = negative
General: WPWYIWLGFIAGLVA — pink = hydrophobic purple = P or G
Alpha-helix: WPWYIWLGFIAGLVA — green = good, orange = bad
Beta-sheet: WPWYIWLGFIAGLVA — green = good
M.W: 1792.1
Net charge: 0 (avg.: 0)
Hydropathy: 16.12 (avg.: 1.075)
Hydropathy Plot: As mention in fig. 9.

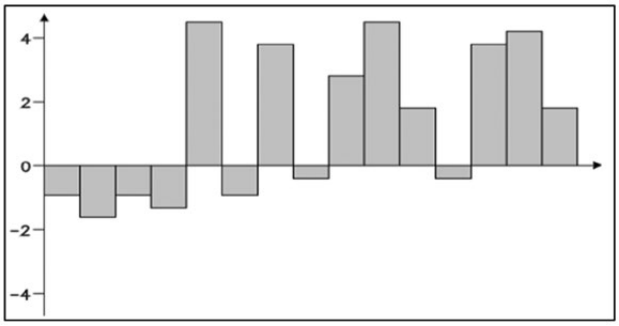

Fig. 9. Hydropathy plot of E. coli peptide (WPWYIWLGFIAGLVA)

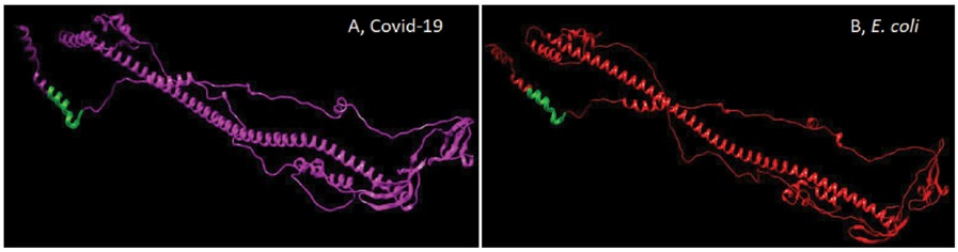

Fig. 10. A part of each 3-dimentianl structure of COVID-19 and E. coli shows the T cell epitope predication. The green color represents the peptide of COVID-19 (WPWYIWLGFIAGLIA) and the peptide of E. coli (WPWYIWLGFIAGLVA)

B Cell epitopes

Spike COVID-19 glycoprotein sequences indicating that they likely possess antigenicity. And these peptides (^{817}IEDLLFNKVTLAD^{830}, ^{912}TQNVLYENQK^{921}, ^{1195}ESLIDLQEL^{1204}) as mention in table 8 have B cell epitope Homology between 13mers peptide of Spike COVID-19 Protein with E. coli, that represent difference Percentage as 15.4%, 20% and 22.2% with identified percentage 84.6%, 80% and 77.8% respectively as mention in table 8, while 3-dimentianl structure of COVID-19 and E. coli shows the B cell epitope predication show in fig. 13.

Table 8
B cell epitope predication between spike COVID-19 and E. coli

No.	Organism	Peptide	Length	Difference	Difference (%)	Identity (%)
1.	COVID-19	IEDLLFNKVTLAD	13	2	15.4	84.6
	E. coli	IEDLLFDKVTIAD	13	2	15.4	84.6
2.	COVID-19	TQNVLYENQK	10	2	20	80
	E. coli	TQQVLSENQK	10	2	20	80
3.	COVID-19	ESLIDLQEL	9	2	22.2	77.8
	E. coli	ESYIDLKEL	9	2	22	77.8

The analysis of COVID-19 peptide with B-cell as mention below peptide sequences, in this study epitope due to net charge is -2, noted the charge peptide have (K) as positive a.a but (E, D, E) have negative value. While find in general formula pink color of a.a (I, L, F, V, A) as hydrophobic but not have hydrophilic properties. while in Alpha-helix find a.a (E, L, K, A) have good interaction site and not has bad interaction site of COVID-19 with E. coli. Hydropathy plot show that most of the amino acid in vaccine are in favorable region.

Length: 13
Charge: IEDLLFNKVTLAD — red = positive, blue = negative
General: IEDLLFNKVTLAD — pink = hydrophobic, purple = P or G
Alpha-helix: IEDLLFNKVTLAD — green = good, orange = bad
Beta- sheet: IEDLLFNKVTLAD — green = good
M.W: 1490.7
Net charge: -2 (avg.: -0.1538)
Hydropathy: 1.87 (avg.: 0.144)
Hydropathy Plot: As mention in fig. 11.

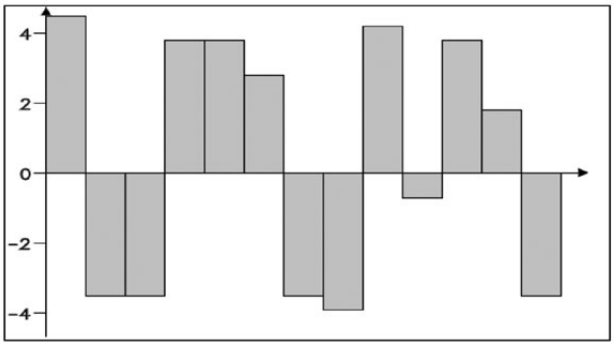

Fig. 11. Hydropathy plot of spike COVID-19 peptide (IEDLLFNKVTLAD)

The analysis of E. coli peptide with B-cell as mention below peptide sequences, in this study epitope due to it net charge is -3, noted the charge peptide have (K) as positive amino acid but (E, D) have negative amino acid. While find in general formula pink color of a.a (I, L, F, V, A) as hydrophobic but not have hydrophilic properties. while in Alpha-helix find a.a (E, L, K, A) have good interaction site and not has bad interaction site of E. coli with COVID-19. Hydropathy plot show that most of the amino acid in vaccine are in favorable region.

Length: 13
Charge: IEDLLFDKVTIAD — red = positive, blue = negative
General: IEDLLFDKVTIAD — pink = hydrophobic, purple = P or G
Alpha-helix: IEDLLFDKVTIAD — green = good, orange = bad
Beta-sheet: IEDLLFDKVTIAD — green = good
M.W: 1491.7
Net charge: -3 (avg: -0.2308)
Hydropathy: 2.84 (avg.: 0.218)
Hydropathy Plot: As mention in fig. 12.

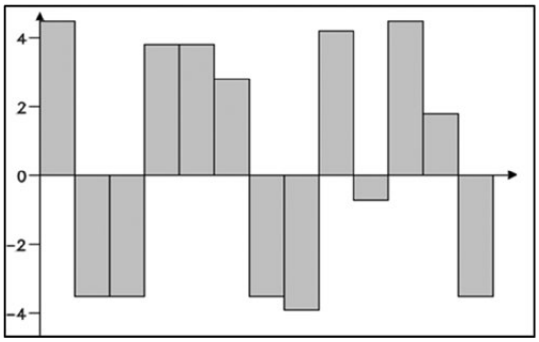

Fig. 12. Hydropathy plot of E. coli peptide (IEDLLFDKVTIAD)

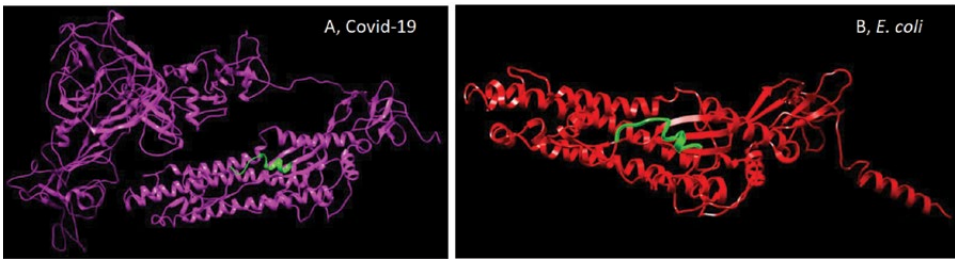

Fig. 13. A part of each 3-dimentianl structure of COVID-19 and E. coli shows the B cell epitope predication, The green color represents the peptide of COVID-19 (IEDLLFNKVTLAD) and the peptide of E. coli (IEDLLFDKVTIAD)

Evaluation of physicochemical parameters

The physicochemical characteristics of the designed MEV concept were predicted using the Expasy ProtParam tool, VaxiJen v2.0, and AllergenFP v.1.0. Spike of COVID-19 have molecular weight 141.17 kDa, and it was made up of 1273 amino acids. while MEV from some bacteria using in this study (E. coli, Pseudomonas aeruginosa, klebsiella pneumonia, Enterobacteria, and Gammaproteobacteria) have Molecular weight 62.62 kDa, 57.12 kDa, 30.02 kDa, 28.10 kDa and 57.80 kDa respectively, while it having amino acid 570, 521, 283, 265 and 363 respectively.

So, the molecular weight is less than 110 kDa, making it an excellent vaccine candidate [31]. The estimated pI of the vaccine was 5.30, 5.9, 4.8, 4.66 and 5.76 respectively so pointing to construct slightly acidic nature. And In vitro modeling suggested a half-life are 7.2 hour, 30 hours, 20 hours, 5.5 hours and 1.4 hours respectively in mammalian reticulocytes. The vaccine's instability index was 31.90, 42.65, 40.44, 43.31 and 45.71 respectively, which are all below the stability threshold of 40 except E. coli is above the stability threshold so is consider as stable protein. The construct was thermostable as evidenced by its aliphatic index of 87.61, 92.84, 91.52, 43.31 and 45.71 respectively while these MEV have Grand average of hydropathicity (GRAVY) -0.054, -0.260, -0.318, -0.295 and -0.219 respectively which highlights hydrophilic nature.

■ DISCUSSION

Since it was discovered at the end of 2019, the 2019 Coronavirus Disease (COVID-19) has been a major cause for concern for public health around the world, Coronavirus 2 causes severe acute respiratory syndrome (SARS-CoV-2) and typically infects the lower airway [32]. As a result of its rapid spread and high mortality rate, the current outbreak of coronavirus disease 2019 (COVID-19) has been declared a global emergency [33]. Developing and using a vaccine could be crucial in either eradicating the virus from human populations or controlling its spread within communities if strict and thorough measures are taken. Since then, there have been significant advances in our understanding of virus biology and its etiology, prompting numerous efforts to address the challenge that has emerged in the current scenario.

COVID-19 has recently been spreading in Wuhan city of China, brought up a lot of questions about how vulnerable humans are to this new pathogen. As was predicted before, unique versions of SARS-CoV will come back. COVID-19 which spread more quickly than the previous versions did. Since COVID-19 had already killed about 646,641 people in 27 July of 2020, a search began for a vaccine against it because the rate of transmission was going up with the development and proliferation of new infectious diseases as COVID-19 variants or hemorrhagic fever virus, immunoinformatic offers a robust and effective strategies for use in advancements in vaccine technology based on epitopes that counteract spreading of COVID-19 epidemic. However, every grows in the number of learning tools of immunoinformatic server, the process of choosing which tools to use for epitope prediction becomes more subjective.

Before putting any stock in a tool's predictions, its performance must be verified. Using an experimentally validated set of COVID-19 epitopes as a benchmark, we were able to determine IEDB server tool for predicting COVID-19 epitopes. Generally demonstrated the most effective combinations for predicting COVID-19 epitope with CD8 and CD4 epitopes respectively, then using another server like NetMHCpanEL4.1 and IEDB.

After that, we used immunoinformatic to identify potentially immunity by T cell epitopes of COVID-19 that useable in the multiepitope of vaccine against the most widely distributed and potentially dangerous variants COVID-19. But in this study recognized alignments of spike glycoprotein of COVID-19 and some bacterial antigens E. coli, Pseudomonas aeruginosa, klebsiella pneumonia, Enterobacteria and Gammaproteobacteria. In this study taken only E. coli with spike COVID-19 due to have more one similarity than the other bacterial antigen as mention in table 2 that have identity percentage 42.43%, so after that identified 3 spike peptides of COVID-19 with 3 peptide of E. coli peptide have epitopes to CD8 (each peptides have 9 a.a) as mention in table 6 the first two peptide having 0% of % difference and 100% of % Identified while the third epitope of it having 11.1% of % difference and 88.9% of % identity to CD8 (MHCI) epitopes. while identified 3 spike peptide of COVID-19 with E. coli peptide have epitopes to CD4 as mention in table 7 the result show 73.3%, 80% and 93.3% of identified % while having difference % are 26.6%, 20% and 6.7% respectively to CD4 (MHCII) epitopes (each peptides have 15 a.a). All of these are hypothesized to be safe for human consumption, contain no allergens, and stimulate the immune system.

Also identified 3 peptides of spike COVID-19 with 3 peptide of E. coli peptide have epitopes to B cell (each peptide length 13 a.a) as mention in table 8 the result show identified % (84.6%, 80% and 77.8%) but it has difference % (15.4%, 20% and 22%), respectively.

These epitopes are promising research targets in development of a vaccine against all known COVID-19 and related strains. The vaccine formulation will be incomplete without the discovery of B cell epitopes. Also isolated CD8 (table 6) and CD4 (table 7) epitope that are: non-toxic, non-allergenic, immunogenic and stable, allowing for more targeted elimination of the mutations that cause each type of concern. Due to the increasing prevalence of infectious variants and worries about declining efficacy of vaccine against the new variants, in order to create booster shots that are more effective against variants, such epitopes could be used.

So, these results similar to Peter J. Eggenhuizen (2022) that using Protein of spike COVID-19 and some of bacteria There found a range of 40–73% identity and 53–73% similarity between both the 15-mer peptide sequences of pathogenic bacteria and COVID-19. The homology regions showed up to a hundred percent resemblance and seventy-seven percent identity when processed as 9mers, a sufficient length for MHCI presenting toCD8+ T cells [34–39].

■ CONCLUSION

A promising method for stopping viral infections is the use of a multi-epitope peptide (MEP) made from overlapping immunodominant epitopes. The predicted vaccine's 3D-structure was modeled using the online server RaptorX. Then modeled structure was refined using the online server for the Galaxy refine tool, so used RAMPAGE to validate the improved 3-D structure.

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