Immunoinformatics Approach for Designing Multiple Epitope-Based Vaccine against Human Metapneumovirus Utilizing its Fusion Protein.

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Abstract

Background

Human Metapneumovirus is a major cause of acute respiratory infections especially in children besides it's responsible for substantial hospitalizations associated with significant morbidity and treatment cost. Hence vaccination is required. Consequently, we aim to predict effective, safe, and universal epitope-based peptides vaccine against the HMPV using its Fusion Protein via the Immunoinformatics approach since there are no licensed vaccines or antiviral treatments yet.

To achieve this goal, various Immunoinformatics databases and web servers, including, the Immune Epitope Database used the Allergen FP v.1.0, and ToxinPred web servers as well as Phyre2 web portal for the modeling of peptide 3D structure and molecular docking study on Cresset Flare software.

Result

According to the results, the peptide GSTVYYPN was the best predicted B-cells epitopes. Moreover, the peptide VIYMVQLPI with population coverage 48.27% in class(C) I, 35.12% in (C)II, and the peptides LIGVYGSSV with 44.03% in C II, YTNVFTLEV with 61.92% in class I were the best-predicted T-cells epitopes that will interact effectively with the MHC I and MHC II molecules respectively.

Conclusions

We recommend the use of them, the highest coverage, and the best -combined allele's bindings of immunogenic multiple peptide vaccines. Also, experimental studies recommend validating the results.

Background

Human metapneumovirus is one of the leading causes of upper and lower respiratory tract infections in human [1–8]. It is a single negative-stranded RNA virus that belongs to the paramyxoviridae family, pneumovirinae subfamily, metapneumovirus genus [1, 7, 9–12]. It is composed of 13000 nucleotides [12], eight genes and, nine proteins, namely: matrix protein, fusion protein, nucleoprotein, phosphoprotein, small hydrophobic protein, attachment protein, transcription elongation factor, RNA synthesis regulatory factor, and RNA dependent RNA polymerase [1, 11–14]. Minor hydrophobic protein, attachment protein, and fusion proteins are glycoproteins that are present on the virion surface [1, 15–18]. Both attachment and fusion glycoproteins are required for the human metapneumovirus to enter the host cell as these proteins enable the attachment and fusion of the virus to the host cells, respectively, and subsequently causing the infection [15–18]. There are four clades of human metapneumovirus genes: A1, A2, B1, B2 [1, 19–23]. Fusion proteins are immunogenic and highly conserved among these different subtypes of human metapneumovirus [24]. The infection caused by human metapneumovirus affects all ages, but it could be severe and fatal in infants, elderly and immunocompromised individuals [5, 7, 12, 25, 31–33]. It also can worsen the condition of asthmatic patients [12, 31–34] and patients with chronic obstructive
pulmonary disease, and it may lead to bronchitis or pneumonia [1, 29, 35, 36, 37] in elderly patients and children. It is responsible for 5 to 15% hospitalization of children with acute respiratory tract infection [10, 26] and 20% of deaths in 2000, with the majority of deaths occurring in sub-Saharan Africa and south of Asia [1, 27]. The infection was transmitted by direct or close contact with infected patients [30, 35]. Although many vaccines and treatments are under investigation, there are no licensed vaccines or antiviral cures against human metapneumovirus yet [9]. The study aims to use in silico approach to determine antigenic peptides from all strains of human metapneumovirus glycoprotein that could be used for peptide-based vaccine design against human metapneumovirus using immunoinformatic tools that are available online. This study is unique because B cells and T cells epitopes are predicted from human metapneumovirus fusion glycoprotein only, which is highly conserved among different subtypes of human metapneumovirus [24], immunogenic [28] and needed for the first step of virus-cell binding interaction [15–18].

**Results**

The predicted B-cells epitopes and their Emini and antigenicity in Table 1 showed the peptide GSTVYYPN was the best peptide, Moreover, the peptide VIYMVQLPI with population coverage 48.27% in class(C) I, 35.12% in (C)II, and the peptides LIGVYGSSV with 44.03% in C II, YTNVFTLEV with 61.92% in class I were the best-predicted T-cells epitopes that will interact effectively with the MHC I and MHC II molecules respectively see table (4)

Table1: Predicted B cell epitope with their eimini and antigenicity score
| Peptide        | Start | End  | Length | Emini Score | Antigenicity Score |
|---------------|-------|------|--------|-------------|--------------------|
| YLEESCTITE    | 23    | 33   | 11     | 0.868       | 1.034              |
| GDVENLTC      | 53    | 60   | 8      | 0.391       | 1.04               |
| DGPSLIK       | 62    | 68   | 7      | 0.878       | 1.021              |
| QLAREEQQIENPROS | 88  | 101  | 14     | 9.75        | 0.976              |
| AAVT          | 116   | 119  | 4      | 0.455       | 1.105              |
| TNEAVSTLGN    | 144   | 153  | 10     | 0.883       | 0.98               |
| KCDI          | 181   | 184  | 4      | 0.523       | 1.09               |
| QFSDNAGITPA   | 206   | 216  | 11     | 0.785       | 0.99               |
| AAPSCS        | 288   | 293  | 6      | 0.414       | 1.105              |
| QGWYRC        | 307   | 311  | 5      | 0.688       | 1.071              |
| GSTVYYPN      | 315   | 322  | 8      | 1.557       | 1.042              |
| KDCETRGDHVF   | 324   | 334  | 11     | 1.166       | 1.015              |
| AAGINVAEQS    | 338   | 347  | 10     | 0.396       | 1.026              |
| ECNIN         | 349   | 353  | 5      | 0.564       | 0.993              |
| TNYPCKV       | 357   | 363  | 7      | 0.992       | 1.091              |
| TGRHP         | 365   | 369  | 6      | 1.974       | 0.965              |
| SYITNQDADTVTIDN | 408 | 422  | 15     | 1.925       | 0.988              |
| SKVEGEQHVI    | 428   | 437  | 10     | 0.836       | 1.056              |
| SFDP          | 445   | 448  | 4      | 1.248       | 1.008              |
| FPEDQFNV      | 451   | 458  | 8      | 1.245       | 1.017              |
| IENSQLALV     | 467   | 474  | 8      | 0.503       | 1.063              |
| SAEKGN        | 483   | 488  | 6      | 2.031       | 0.918              |

Table 2: The predicted T-cell epitopes that interact with MHCI alleles with their percentile rank and IC50
| Percentile | Ic50  | Allele          | End | Start | Peptide         |
|-----------|-------|-----------------|-----|-------|-----------------|
| 0.6       | 285.95| HLA-B*40:02     | 28  | 20    | KESYLEESC       |
| 0.17      | 93.0  | HLA-C*14:20     | 30  | 22    | SYLEESCST       |
| 0.48      | 44.36 | HLA-A*02:01     | 31  | 23    | YLEESCSTI       |
| 0.78      | 79.74 | HLA-A*02:06     |     |       |                 |
| 0.68      | 421.61| HLA-C*03:03     |     |       |                 |
| 0.2       | 428.87| HLA-C*05:01     |     |       |                 |
| 0.1       | 40.13 | HLA-C*12:30     |     |       |                 |
| 0.37      | 262.58| HLA-C*14:02     |     |       |                 |
| 1.9       | 226.09| HLA-A*02:01     | 45  | 37    | SVLRTGWYVT      |
| 1.6       | 415.33| HLA-B*27:05     | 47  | 39    | LRTGWYTNV       |
| 0.11      | 365.35| HLA-C*07:01     |     |       |                 |
| 0.36      | 203.72| HLA-A*24:02     | 48  | 40    | RTGWYTNVF       |
| 0.9       | 243.06| HLA-A*30:02     |     |       |                 |
| 0.02      | 6.61  | HLA-A*32:01     |     |       |                 |
| 0.78      | 171.06| HLA-B*15:01     |     |       |                 |
| 0.22      | 47.72 | HLA-B*58:01     |     |       |                 |
| 0.23      | 436.9 | HLA-C*15:02     |     |       |                 |
| 0.17      | 46.75 | HLA-A*23:01     | 50  | 42    | GWYTNVFTL       |
| 0.3       | 171.89| HLA-A*24:02     |     |       |                 |
| 0.05      | 209.73| HLA-C*07:02     |     |       |                 |
| 0.23      | 138.87| HLA-C*14:02     |     |       |                 |
| 0.17      | 63.41 | HLA-A*01:01     | 52  | 44    | YTNVFTLEV       |
| 0.43      | 39.42 | HLA-A*02:01     |     |       |                 |
| 0.55      | 49.99 | HLA-A*02:06     |     |       |                 |
| 0.15      | 14.51 | HLA-A*68:02     |     |       |                 |
| 0.2       | 111.42| HLA-C*12:03     |     |       |                 |
| 0.01      | 16.47 | HLA-C*15:02     |     |       |                 |
|    |    |    |    |    |    |
|----|----|----|----|----|----|
| 0.09 | 25.8 | HLA-B*40:01 | 58 | 50 | LEVGDVENL |
| 0.85 | 431.91 | HLA-B*40:02 |    |    |    |
| 0.28 | 27.18 | HLA-B*07:02 | 71 | 63 | GPSLIKTELE |
| 0.75 | 364.35 | HLA-A*30:01 | 81 | 73 | LTKSALREL |
| 0.15 | 102.67 | HLA-B*44:02 | 103 | 95 | IENPRQSRF |
| 0.01 | 4.42 | HLA-B*07:02 | 105 | 97 | NPRQSRFVL |
| 0.19 | 72.33 | HLA-B*07:02 |    |    |    |
| 0.68 | 68.53 | HLA-A*02:06 | 107 | 99 | RQSRFVLGA |
| 0.87 | 478.32 | HLA-A*30:01 |    |    |    |
| 1.7 | 479.78 | HLA-B*27:05 |    |    |    |
| 0.14 | 77.11 | HLA-C*14:02 | 110 | 102 | RFVLGAIAL |
| 1.9 | 279.92 | HLA-A*02:06 | 111 | 103 | FVLGAIALG |
| 0.58 | 54.08 | HLA-A*02:01 | 112 | 104 | VLAGIALGV |
| 1.8 | 261.15 | HLA-A*02:06 |    |    |    |
| 1.9 | 302.3 | HLA-A*02:06 | 134 | 126 | KTIRLESEV |
| 0.04 | 58.71 | HLA-C*15:02 |    |    |    |
| 1.2 | 128.79 | HLA-A*02:06 | 155 | 147 | AVSTLNGGV |
| 0.99 | 219.15 | HLA-A*68:02 |    |    |    |
| 2.4 | 439.34 | HLA-A*02:06 | 157 | 149 | STLNGVRV |
| 0.84 | 156.59 | HLA-A*68:02 |    |    |    |
| 0.58 | 241.0 | HLA-A*30:01 | 162 | 154 | GVRVLATAV |
| 1.9 | 226.09 | HLA-A*02:01 | 165 | 157 | VLATAVREL |
| 1.6 | 216.61 | HLA-A*02:06 | 196 | 188 | KMAVSFSQF |
| 0.78 | 371.42 | HLA-A*23:01 |    |    |    |
| 0.69 | 458.54 | HLA-A*24:02 |    |    |    |
| 0.26 | 237.52 | HLA-A*32:01 |    |    |    |
| 0.05 | 11.33 | HLA-B*15:01 |    |    |    |
| 0.34 | 81.96 | HLA-B*58:01 |    |    |    |
| 0.55 | 263.06 | HLA-B*35:01 | 197 | 189 | MAVSFSQFN |
| 1.5 | 370.18 | HLA-A*03:01 | 198 | 190 | AVSFSQFNR |
|     |          | HLA-A*11:01 | 199 | 191 | VSFQFNRRR |
|-----|----------|-------------|-----|-----|-----------|
| 0.13| 22.16    | HLA-A*31:01 |     |     |           |
| 0.17| 14.32    | HLA-A*68:01 |     |     |           |
| 0.69| 102.27   | HLA-A*11:01 | 199 | 191 | VSFSQFNRRR |
| 0.16| 13.62    | HLA-A*31:01 |     |     |           |
| 0.72| 95.2     | HLA-A*68:01 |     |     |           |
| 0.61| 393.3    | HLA-A*24:02 | 200 | 192 | SFSQFNRRRF |
| 0.06| 137.33   | HLA-C*06:02 | 201 | 193 | FSQFNRRRFL |
| 0.13| 60.38    | HLA-C*12:03 |     |     |           |
| 0.08| 34.12    | HLA-C*12:03 | 204 | 196 | FNRRFLNVV |
| 0.27| 72.0     | HLA-B*27:05 | 206 | 198 | RRFLNVVRQ |
| 0.15| 38.7     | HLA-A*23:01 | 207 | 199 | RFLNVVRQF |
| 0.16| 106.71   | HLA-A*24:02 |     |     |           |
| 0.25| 215.15   | HLA-A*32:01 |     |     |           |
| 0.56| 490.25   | HLA-C*14:02 |     |     |           |
| 0.59| 253.25   | HLA-A*30:01 | 211 | 203 | VVRQFSDNA |
| 0.58| 54.22    | HLA-A*02:06 | 214 | 206 | RQFSDNSAGI |
| 1.3 | 301.73   | HLA-B*15:01 |     |     |           |
| 1.5 | 407.1    | HLA-B*27:05 |     |     |           |
| 0.35| 111.38   | HLA-C*05:01 | 215 | 207 | FSDNAGITP |
| 1.6 | 416.65   | HLA-A*68:02 | 217 | 209 | DNAGITPAI |
| 0.35| 127.59   | HLA-C*03:03 | 219 | 211 | AGITPAISL |
| 0.52| 450.44   | HLA-C*14:02 | 221 | 213 | ITPAISLDL |
| 0.27| 85.95    | HLA-B*35:01 | 222 | 214 | TPAISLDLM |
| 1.7 | 166.24   | HLA-A*31:01 | 248 | 240 | QIKLMLENR |
| 1.7 | 311.74   | HLA-A*68:01 |     |     |           |
| 1.3 | 465.82   | HLA-B*07:02 | 250 | 242 | KLMLENRAM |
| 0.99| 251.82   | HLA-B*15:01 |     |     |           |
| 0.07| 40.02    | HLA-C*14:02 |     |     |           |
| 0.38| 33.58    | HLA-A*02:01 | 251 | 243 | LMLENRAMV |
| Score | Value  | Allele          | Position 1 | Position 2 | Peptide     |
|-------|--------|-----------------|------------|------------|-------------|
| 0.23  | 17.43  | HLA-A*02:06     |            |            | MLENRAMVR   |
| 0.49  | 414.98 | HLA-C*12:03     |            |            |             |
| 1.8   | 192.75 | HLA-A*31:01     | 252        | 244        | RAMVRVKGF   |
| 1.2   | 167.51 | HLA-A*68:01     |            |            |             |
| 0.93  | 326.77 | HLA-B*07:02     | 256        | 248        |             |
| 0.11  | 41.4   | HLA-B*08:01     |            |            |             |
| 0.58  | 185.15 | HLA-B*57:01     |            |            |             |
| 0.14  | 28.29  | HLA-A*30:01     | 258        | 250        | MVRKGFGLI   |
| 0.31  | 129.72 | HLA-B*08:01     |            |            |             |
| 0.6   | 268.16 | HLA-A*30:01     | 260        | 252        | RRKFGILIGV  |
| 0.08  | 25.05  | HLA-B*27:05     |            |            |             |
| 0.74  | 74.41  | HLA-A*02:06     | 262        | 254        | KGFGILIVY   |
| 1.2   | 307.12 | HLA-A*29:02     | 263        | 255        |             |
| 0.82  | 261.58 | HLA-A*30:02     |            |            |             |
| 0.44  | 105.26 | HLA-A*03:01     | 269        | 261        | GVYGSVIY    |
| 0.7   | 102.81 | HLA-A*11:01     |            |            |             |
| 0.17  | 22.0   | HLA-A*29:02     |            |            |             |
| 0.31  | 87.83  | HLA-A*30:02     |            |            |             |
| 0.78  | 167.62 | HLA-B*15:01     |            |            |             |
| 0.65  | 350.49 | HLA-B*35:01     |            |            |             |
| 0.08  | 44.78  | HLA-C*14:02     | 270        | 262        | VYGSSVIYM   |
| 2.7   | 427.15 | HLA-A*02:01     | 271        | 263        | YGSSVIYMV   |
| 2.1   | 359.13 | HLA-A*02:06     |            |            |             |
| 0.38  | 46.96  | HLA-A*68:02     |            |            |             |
| 0.2   | 115.96 | HLA-C*12:03     |            |            |             |
| 1.3   | 137.15 | HLA-A*02:01     | 275        | 267        | VIYMVQLPI   |
| 1.7   | 231.88 | HLA-A*02:06     |            |            |             |
| 0.11  | 79.87  | HLA-A*32:01     |            |            |             |
| 0.28  | 182.9  | HLA-C*14:02     |            |            |             |
| 0.23  | 456.3  | HLA-C*15:02     |            |            |             |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 0.05 | 15.34 | HL:0A-A*23:01 | 276 | 268 | IYMVQLPIF |
| 0.02 | 15.62 | HLA-A*24:02 |   |   |   |
| 0.16 | 88.99 | HLA-C*14:02 |   |   |   |
| 1.3  | 133.11 | HLA-A*02:01 | 276 | 270 | MVQLPIFGV |
| 0.25 | 20.62 | HLA-A*02:06 |   |   |   |
| 0.07 | 7.67  | HLA-A*68:02 |   |   |   |
| 0.21 | 16.39 | HLA-A*02:06 | 279 | 271 | VQLPIFGVI |
| 1.7  | 472.81 | HLA-B*15:01 |   |   |   |
| 0.75 | 296.91 | HLA-B*58:01 | 309 | 301 | CLLREDQGW |
| 1.4  | 360.17 | HLA-A*30:02 | 310 | 302 | LLREDQGWY |
| 1.3  | 314.49 | HLA-B*15:01 |   |   |   |
| 0.49 | 278.92 | HLA-B*18:01 | 334 | 326 | CETRGDHFV |
| 0.26 | 80.04  | HLA-B*35:01 | 340 | 332 | HVFCDTAAG |
| 0.4  | 51.76  | HLA-A*68:02 | 344 | 336 | DTAAGINVA |
| 1.8  | 264.17 | HLA-A*02:06 | 370 | 362 | KVSTGRHPI |
| 0.3  | 84.76  | HLA-A*30:01 |   |   |   |
| 0.21 | 156.17 | HLA-A*32:01 |   |   |   |
| 0.59 | 171.75 | HLA-B*07:02 |   |   |   |
| 0.32 | 483.17 | HLA-B*39:01 | 375 | 367 | RHPISMVAL |
| 0.04 | 385.56 | HLA-B*48:01 |   |   |   |
| 0.23 | 146.04 | HLA-C*14:02 |   |   |   |
| 0.49 | 216.61 | HLA-B*35:01 | 376 | 368 | HPISMVALS |
| 0.58 | 53.87  | HLA-A*02:06 | 378 | 370 | ISMVALSPL |
| 0.91 | 220.25 | HLA-B*15:01 |   |   |   |
| 0.62 | 209.97 | HLA-B*58:01 |   |   |   |
| 0.09 | 165.86 | HLA-C*15:02 |   |   |   |
| 0.14 | 54.37  | HLA-A*68:02 | 380 | 372 | MVALSPLGA |
| 2.2  | 374.62 | HLA-A*02:06 | 381 | 373 | VALSPLGAL |
| 1.2  | 394.88 | HLA-B*07:02 |   |   |   |
| 0.06 | 9.92   | HLA-C*03:03 |   |   |   |
Table 4: the predicted MHC1, MHC11, and the MHC combined peptide are having the highest percent in population coverage

| MHC I Peptides | The coverage | MHC II peptides | The coverage | MHC combined | The coverage |
|----------------|--------------|-----------------|--------------|--------------|--------------|
| YTNVFTLEV      | 61.92%       | IKLMLLENRA      | 48.63%       | YTNVFTLEV    | 61.92%       |
| YLEESCSTI      | 57.06%       | LIGVYGSSV       | 44.03%       | YLEESCSTI    | 57.06%       |
| VIYMQVQLPI     | 48.27%       | VIYMQVQLPI      | 35.12%       | IKLMLLENRA   | 48.63%       |
| Epitope set    | 99.52%       |                 | 81.94%       |              | 99.91%       |
Table 5: The predicted Allergicity, toxicity, and the molecular docking scores of the predicted peptides with MHC IA, MHC IB, and MHC II molecules.

| The Peptides      | MHC IA Docking score (6AM5) | MHC IB Docking score (5TXS) | MHC II Docking score (5NI9) | The Allergenicity | The Toxicity |
|-------------------|-----------------------------|-----------------------------|----------------------------|--------------------|--------------|
| YTNVFTLEV         | Not docked                  | Not docked                  | —                           | Non-allergen       | Non-Toxin    |
|                   |                             |                             |                             |                    |              |
| YLEESCSTI         | Not docked                  | Not docked                  | —                           | Probable allergen  | Non-Toxin    |
|                   |                             |                             |                             |                    |              |
| VIYMVQLPI         | -4.893                      | -4.235                      | —                           | Probable allergen  | Non-Toxin    |
|                   |                             |                             |                             |                    |              |
| IKLMLLENRA        | —                           | —                           | Not docked                  | Probable allergen  | Non-Toxin    |
|                   |                             |                             |                             |                    |              |
| LIGVYGSSV         | —                           | —                           | -6.244                      | Non-allergen       | Non-Toxin    |
|                   |                             |                             |                             |                    |              |
| VIYMVQLPI         | —                           | —                           | -6.556                      | Probable allergen  | Non-Toxin    |
|                   |                             |                             |                             |                    |              |
| YTNVFTLEV         | Not docked                  | Not docked                  | Not docked                  | Non-allergen       | Non-Toxin    |
|                   |                             |                             |                             |                    |              |
| YLEESCSTI         | Not docked                  | Not docked                  | Not docked                  | Probable allergen  | Non-Toxin    |
|                   |                             |                             |                             |                    |              |
| IKLMLLENRA        | Not docked                  | Not docked                  | Not docked                  | Probable allergen  | Non-Toxin    |
|                   |                             |                             |                             |                    |              |

Discussion

We highlighted our most promising antigenic peptide of the B-cells epitopes GSTVYYPN (Table 1). And for the T-cells epitopes the best MHC I Peptides are YTNVFTLEV, YLEESCSTI and VIYMVQLPI (Table 2).

Moreover, the best MHC II peptides are IKLMLLENRA, LIGVYGSSV and VIYMVQLPI (Table 3) the epitope-based peptide vaccines that include the B-cells and T-cells epitopes are well antigenic [38] and could be produced simply [39]. comparing our study with other studies predicted immunogenic epitope for the Design of Epitope-Based HMPV Vaccines (Rock et al., 2011) in terms of agreement MHC class 1 binding with 3 conventional alleles HLA-A*01:01, HLA-A*02:01, and with HLA-B*07:02 alleles using (BIMAS) bioinformatics analysis tool [40] Our finding for MHC 1 Peptides binds 17 alleles HLA-A*01:01, HLA-A*02:01, HLA-A*02:02, HLA-A*02:06, HLA-A*03:03, HLA-A*05:01, HLA-C*12:30 and HLA-C*14:02, HLA-A*02:01, HLA-A*02:06, HLA-A*32:01, HLA-C*14:02, and HLA-C*15:02 (Table 2).
Moreover, the best MHC II peptides binds with the alleles HLADRB1*01:01, HLA-DRB4*01:01, HLA-DRB1*04:01, HLA-DRB1*11:01, HLADRB1*15:01, HLA-DRB1*04:05, HLA-DRB5*01:01 and HLA-DRB1*09:01, (Table 3).

The predicted peptides for MHC I and MHC II have a highest population coverage. For MHC I, MHC II peptides the percentage were 99.52%, 81.94%, respectively and for MHC combined was 99.91% (Table 4).

**Conclusion**

The study led to the prediction of effective and safe epitope-based peptides vaccine against the HMPV using its Fusion Protein via the Immunoinformatics approach. The peptide **GSTVYYPN** was the best predicted B-cells epitopes. Moreover, the peptide **VIYMVQLPI** and the peptides **LIGVYGSSV, YTNVFTELV** were the best-predicted T-cells epitopes that will interact effectively with the MHC I and MHC II molecules, respectively. Consequently, we recommend the use of them as combined multiple peptide vaccines. Also, experimental studies recommended to validate the results.

**Methods**

**Retrieval of the targeted sequences**

A total of 182 Human Metapneumovirus Fusion Protein sequences were obtained from the NCBI database [41] as the FASTA format in August 2018. The accession numbers of the obtained sequences with the area and date of the collection were listed in Table 6.
Table 6  
*the retrieved Human Metapneumovirus Fusion Protein sequences with their Accession number, Date and area of Collection*

| Accession number | Date of collection | country  |
|------------------|--------------------|----------|
| YP_012608        | 2004               | USA      |
| BBB35016         | 2015               | Japan    |
| BBB35015         | 2015               | Japan    |
| BBB35014         | 20/3/2015          | Japan    |
| BBB35013         | 2015-03-16"        | Japan    |
| BBB35012         | 2015-03-16"        | Japan    |
| BBB35011         | 2015-02-27         | Japan    |
| BBB35010         | 2015-02-21         | Japan    |
| BBB35009         | 2015-02-21         | Japan    |
| BBB35008         | 2015-02-21"        | Japan    |
| BBB35007.1       | 2014-04-12         | Japan    |
| BBB35006.1       | 2014-04-13"        | Japan    |
| BBB35005.1       | 2014-04-05         | Japan    |
| BBB35004.1       | 2013-07-08"        | Japan    |
| BBB35003.1       | 2013-06-22"        | Japan    |
| BBB35002.1       | 2013-06-24         | Japan    |
| BBB35001.1       | 2013-06-24         | Japan    |
| BBB35000.1       | 2013-04-30         | Japan    |
| BBB34999.1       | 2013-04-16         | Japan    |
| BBB34998.1       | 2013-04-08"        | Japan    |
| BBB34997.1       | 2013-03-25         | Japan    |
| BBB34996.1       | 2013-01-21         | Japan    |
| BBB34995.1       | 2015-03-02         | Japan    |
| BBB34994.1       | 2014-08-04         | Japan    |
| Accession number | Date of collection | Country |
|------------------|--------------------|---------|
| BBB34992.1       | 2013-06-25         | Japan   |
| BBB34991.1       | 2013-06-24         | Japan   |
| BBB34990.1       | 2013-06-22         | Japan   |
| ANW38002.1       | 2011               | Croatia |
| ANW38000.1       | 2013               | Croatia |
| ANW37997.1       | 2012               | Croatia |
| ANW37998.1       | 2012               | Croatia |
| ANW37996.1       | 2012               | Croatia |
| ANW37993.1       | 2011               | Croatia |
| ANW37992.1       | 2011               | Croatia |
| ANW37991.1       | 2011               | Croatia |
| ANW37990.1       | 2011               | Croatia |
| ANW37989.1       | 2011               | Croatia |
| AII17595.1       | 10-Aug-2011        | South Korea |

**Determinant of the conserved regions**

The obtained Sequences subjected to the multiple alignment tests via the CLUSTALW algorithm [42] on BioEdit software [43] version 7.0.9.1 to identify the conserved regions.

**B-Cells Epitopes Prediction**

The linear Epitope Prediction tool BepiPred-test on the Immune Epitope Database (IEDB) [44] (figure).

The epitopes were predicted at a default threshold value of (0.4) from the conserved region in the Human Metapneumovirus Fusion Protein sequences that obtained from the multiple sequences, alignment. The prediction performed using the Markov model [45]. The results listed in Table 1.

**The Surface Accessibility Prediction**

The Emini surface accessibility prediction tool [43] on the IEDB [46] used to predict the surface accessibility with a default threshold value for each conserved region (Fig. 9). The results are listed in Table 1.

**The Antigenic Sites Prediction**
The Kolaskar and Tongaonker antigenicity tool on IEDB [47] used for the prediction of the antigenic sites within the Human Metapneumovirus Fusion Protein sequences at a default threshold value of 1.04. The results listed in Table 1, (Fig. 10). For the whole first alignment, sequences see (Fig. 11).

**T-Cell Epitopes Prediction**

The prediction of the cytotoxic T-cell epitopes performed by using the Major Histocompatibility Complex class I (MHC I) binding prediction tool on IEDB [48]. The epitopes' length adjusted at 9. The conserved epitopes that bind with various HLA alleles at score equal or less than 1.0 percentile rank and 500 IC50 selected for further analysis. Moreover, the prediction of T-cell helper epitopes performed by using the Major Histocompatibility Complex class II (MHC II) binding prediction tool of IEDB [49]. The results listed in Table 3.

**The Population Coverage Prediction**

The prediction of epitopes binding with various MHC I and MHC II alleles that cover the world population was performed by using the population coverage tool on the IEDB [50]. The results are listed in Table 4.

**The Peptides Allergicity and Toxicity Prediction**

The AllergenFP v.1.0 [47] web servers used to predict the Allergenicity. Furthermore, ToxinPred web server [48] used to predict the Toxicity. The results listed in Table 5.

**The 3D Structure Modeling and Visualization**

The 3D structure of the Human Metapneumovirus Fusion Protein modeled by using the Phyre2 web portal [49] and 3D structure the predicted peptides we modeled by using PEP-FOLD 3.5 web server [50]. The modeled 3D structures visualized by Chimera 1.8 software [51]. The results presented in Figs. 1, 2, and .

**5 The Molecular Docking Study**

The predicted epitopes were docked with MHC I and MHC II molecules. The Protein Databank [52] was used to obtain the 3D structures of MHC I and MHC II. The 3D structures (PDBIDs: 6AM5, 5TXS, and 5NI9 for MHC IA, MHC IB, and MHC II respectively) were downloaded in PDB format. The structures were prepared, minimized for the docking process, and the molecular docking calculation performed by using Cresset Flare software [53]. The results listed in Table 5 and showed in Figs. 4,5,6, and 7.

**Declarations**

**Ethics approval and consent to participate**
Consent for publication

Not applicable

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that having no conflict of interest.

Funding

Authors declare that there's no fund available for this study

Authors' contributions

All authors contributed in this work as follows:

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**Tables**

Due to technical limitations, table 3 is only available as a download in the Supplemental Files section.

**Figures**
Figure 1

The position of predicted B-cells peptide GSTVYYPN (purple) on the 3D structure of human Metapneumovirus Fusion Protein

Figure 2

The position of predicted MHC I peptides (purple) on the 3D structure of human Metapneumovirus Fusion Protein

Figure 3

The position of predicted MHC II peptides (purple) on the 3D structure of human Metapneumovirus Fusion Protein
Figure 4

The 3D interaction between the predicted peptide (violet) VIYMVQLPI with MHC IA molecule

Figure 5

The 3D interaction between the predicted peptide VIYMVQLPI (violet) with the MHC IB molecule
Figure 6

The 3D interaction between the predicted peptide LIGVYGSSV (violet) with the MHC II molecule

Figure 7

The 3D interaction between the predicted peptide VIYMVQLPI (violet) with MHC II molecule
Figure 8

Bipered linear prediction diagram, where green color referred to predicted peptides
Figure 9

Emini Surface Accessibility Prediction Results, where green color referred to predicted peptides.
Figure 10

Kolaskar & Tongaonkar Antigenicity test result, where green color referred to predicted peptides

Figure 11

The result of the ready BioEdite alignment server for HMNP the partial part of the whole sequence.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table3.docx