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Research article

In silico comparative analysis of SARS-CoV-2 nucleocapsid (N) protein using bioinformatics tools

Mehmet Emin Uras[1]

[1] *Marmara University, Faculty of Science & Arts, Department of Biology, 34722, Goztepe, Istanbul, Turkey*

Abstract

The world has been encountered to one of the biggest pandemics that causing by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is placed in the Beta-CoV genus in the Coronaviridae family. N protein is one of the crucial structural proteins of SARS-CoV-2 that binds to the genome thereby generating helical ribonucleoprotein core. It is involved in viral transcription/replication, translation, and viral assembly after entering the host cell through interacting with host proteins. N protein sequences of SARS-CoV-2 and taxonomically related CoVs are examined using bioinformatics tools and approaches including sequence alignment, sequence and phylogenetic analyzes, and predicting of putative N-Glycosylation and phosphorylation positions and also predictions and comparative analyzes are performed on 3D structures of N proteins from SARS-CoV-2 related CoVs through using of some of applied bioinformatics analyzes. Results of mega BLAST search revealed that the most similar N protein sequence to SARS-CoV-2 is Bat-CoV RaTG13 N protein sequence in the taxonomically related CoVs. SARS-CoV-2 is grouped with SARS, pangolin, civet and bat CoVs (RATG13, SL ZC45 and SL ZXC21) in N protein, nucleotide and protein based ML phylogenetic trees. Some of SARS-CoV-2 N proteins were showed divergence from other SARS-CoV-2 N proteins analyzed due to amino acid substitutions detected in SARS-CoV-2 N proteins samples in phylogenetic trees. The highest amino acid substitutions were detected in Richmont/USA (QJA42209.1) and Greece (QIZ16579.1) samples, with 2 and 3 place substitutions, respectively. By domain analyzes, three domains were detected as Corona_nucleocora (Pfam), N terminal CoV RNA-binding domain (HAMAP) and C terminal N protein dimerization domain (HAMAP). Possible N-glycosylation positions of SARS-CoV-2 N protein were predicted at two positions. Assessments of possible serine, threonine and tyrosine phosphorylations were found to be at 100 positions, 34 of them were higher than 80% possibility. 3D structure analysis based on TM scores revealed that although the results of 3D structure analysis were shown consistency with the taxonomy of the CoVs, the 3D structures of SARS-CoV-2 N protein and taxonomically related CoVs were not at the same fold.

Keywords: *3D structure; bioinformatics; coronavirus; COVID-19; SARS-CoV-2; viral proteins*

1. Introduction

By the end of 2019, the world has been encountered to one of the biggest pandemics that caused by severe acute respiratory syndrome coronavirus 2 (2019-nCoV or SARS-CoV-2). In December 2019, WHO authorities were informed by Chinese authorities for a new pneumonia infection, mainly resembling viral pneumonia, in Wuhan/China (Genc, 2020; Wu et al., 2020). After the first examinations, the cause of the infection was diagnosed as a novel CoV (SARS-CoV-2); thereafter, named as COVID-19. Meanwhile 282 cases and 6 deaths on January 21, 2020, and following 3,349,786 cases and 238,628 deaths on May 3, 2020 and 79,062,802 cases and 1,751,311 deaths on December 27, 2020 were reported by WHO in all around the world (WHO, 2020b, 2020a, 2020c).

SARS-CoV-2 is a member of Beta-CoV genera from

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* Corresponding author.
E-mail address: mehmeteminuras@gmail.com (M. E. Uras).
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family *Coronaviridae*, like severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) (Ye et al., 2020). Genome structure of SARS-CoV and MERS-CoV were extensively studied after the pandemics occurred during the periods of 2002-2003 and 2012-2015, respectively (De Wit, et al., 2016). The first genome sequence of SARS-CoV-2, sampled from a patient from Wuhan/China, were submitted to NBCI GenBank at the beginning of January 2020 with accession number of MN908947 (Wu et al., 2020).

The genome analyzes of the Beta-CoVs revealed that they have a large single-stranded, approximately ~30 kb-bp long positive-sense RNA genome (+ssRNA). The strand has a poly-A tail at 3′ and cap at 5′. The two thirds of the genome encodes a non-structural protein named 1a/1b (ORF1a/b) polyprotein having a role in construction of replication/transcription complex (Chen, et al., 2020). The remaining part of genome encodes structural proteins including spike glycoprotein (S protein), envelope glycoprotein (E protein), and membrane (M protein) and nucleocapsid (N protein) proteins in a 5′ to 3′ order. Additionally, some proteins are also encoded by the structural proteins which are involved in immune response of host (De Wit et al., 2016)

The S protein of CoVs is responsible for recognition and entry into the host. It binds to the receptor protein of the host, initiating membrane fusion. Angiotensin-converting enzyme 2 (ACE2) bound by SARS-CoV and SARS-CoV-2, and dipeptidyl peptidase 4 (DPP4 or CD26) bound by MERS-CoV are found as receptors on the hosts (De Wit et al., 2016). E protein is an integral membrane protein and is crucial for virus assembly and budding. It enhances virus budding (Neuman et al., 2011; Nieto-Torres et al., 2011). M protein is also an integral membrane protein and is vital in producing new viruses. It binds to the membrane allowing newly produced viruses to scatter by budding (Neuman et al., 2011).

N protein of CoVs is 45-50 KDa phosphoprotein that is involved in: i) virus assembly and also viral genome +ssRNA replication and/or transcription, and ii) viral mRNA translation. N protein interacts with +ssRNA, M protein and N protein itself (Hogue & Machamer, 2008). It binds to viral genome and other N proteins in order to generate helical ribonucleoprotein core. During the process, it also acts as RNA chaperone. N protein enters into the host cell with the viral genome and interacts with host cell proteins. The structural organization analyzes of SARS-CoVs revealed that N protein has two non-interacting structural domains, of are as N-terminal RNA-binding domain between 45-181 residues and C-terminal dimerization domain between 248-365 residues (Chang et al., 2006). Additionally, SARS-CoV M protein binds to N protein between amino acids located at 211–254 and/or 168–208 positions and these regions play important roles in N protein-protein interactions (He et al., 2004; Fang et al., 2006). N protein may undergo some post-translational modifications, including sumoylation, proteolytic cleavage, ADP-ribosylation and phosphorylation (Fung & Liu, 2018). Phosphorylation can alter function of N proteins during viral life-cycle. Phosphorylation of N protein usually occurs in serine-arginine rich region or domain 3 in the protein. Phosphorylation has some effects on N protein including subcellular localization and antigenic specificity (Chang et al., 2006; Huang et al., 2015). Also, N protein has some additional functions such as nucleocytoplasmic shuttling, inhibition of S phase progress of the host and development of viral infection (Satija & Lal, 2007; Fung & Liu, 2018).

Most of CoVs N protein have epitopic sites that can be used to diagnose the infection. For instance, in SARS-CoV, the site between 371–407th amino acids in C terminus is identified as most antigenic region (Li et al., 2003). The N protein is a major structural component of CoVs and one of the most abundant viral protein produced in the host cell; therefore, can be used as antigenic protein for diagnostic purposes, and efforts in developing medicine and/or vaccine and preventing the infection. Additionally, high level of antibodies against N protein is reported in SARS-CoV patients by several researchers (Satija & Lal, 2007).

This study is aimed to perform comparative bioinformatics analysis of N protein of CoVs in order to determine various properties of SARS-CoV-2 N protein. Additionally, 3D structures of SARS-CoV-2 N protein are also generated and analyzed.

2. Materials and methods

2.1. Sequence retrieving

40 nucleotide RNA and polypeptide sequences of SARS-CoV-2 N protein were retrieved from NCBI GenBank. Coding sequences (CDS) of N protein in the viral genome were retrieved from features of CDS option except only CDS of N protein from pangolin (Zhang et al., 2020), retrieved manually from viral genome MT084071. The accession numbers of protein sequences and viral genomes, the CDS positions of N proteins, the lengths of proteins and CDS, and the origins of the countries of the viral genomes were shown in Supplementary Table 1 (S-Table1). When selecting sequences, the countries considered as epicenters of the pandemic are preferred. The distribution of the sequences by countries is of as following; 13 from USA, 9 from China, 3 from Japan, 2 from Vietnam, 2 from Italy, 2 from Colombia, 1 from each of Israel, Iran, Greece, Brazil, Spain, India, Australia and Turkey.

Additionally, 42 coding nucleotide and protein sequences from the other CoVs were also retrieved from NCBI GenBank using the same method. The sources of the sequences, the accession numbers of the N protein sequences and the viral genomes, the CDS positions of N proteins, the lengths of the proteins and CDS were shown S-Table 2. The selected other CoV sequences are of as following; 16 from bat CoVs, 6 from pangolin CoVs (except for the protein sequence of pangolin CoV isolate MP789), 2 from civet CoV, 1 from each of rabbit and camel, 2 from human beta CoV, 1 from human enteric CoV, 3 from human MERS-CoV, 7 from human SARS and finally, 3 from avian infectious bronchitis virus (Avian IBV) Gama-CoV.

2.2. Sequence alignment and analyses

All the 81 nucleotide and protein sequences retrieved were aligned as two data sets by using BioEdit software v7.2.5 (Hall, 1999) with Clustal W multiple alignment application (Thompson, Higgins, & Gibson, 1994). Separately, 19 Sarbecoviruses selected were analyzed for sequence variations. The SARS-CoV-2 N protein percentage identities of NCBI database (NCBI, 2020) were evaluated by using BLASTP suite (Basic Local Alignment Search Tool, protein-protein Blast) with blastp option. To compare SARS-CoV-2 N proteins, the percentage of identities and cover analysis were conducted by using SARS-CoV-2 isolate N protein sequences (YP_009724397.2 Wuhan-Hu-1 and QHD43423.2 Shanghai/

China). Domain analyzes of SARS-CoV-2 N proteins were performed using Pfam 32.0 database (El-Gebali et al., 2019) and HAMAP database (Pedruzzi et al., 2015). Tajima's test of neutrality (Tajima, 1989) was used in MEGA X software (Kumar et al., 2018) for the calculating nucleotide diversities (π), the numbers of segregating sites (S), and the Tajima's test statistic (D) values. 19 Sarbecovirus sequences, previously used for alignment were also used to perform the Tajima's test of neutrality

2.3. Phylogenetic analyzes

Phylogenetic analyzes were inferred by using both of the nucleotide and protein sequences. The nucleotide sequence based phylogenetic tree was constructed using Maximum Likelihood (ML) method and Tamura-Nei model. The nucleotide sequence based phylogenetic tree were included 82 nucleotide sequences and 1586 positions in the final data set. The two N-protein amino acid sequence based trees were also online server used for the putative phosphorylation positions (Blom et al., 2004) (http://www.cbs.dtu.dk). The putative phosphorylation positions were predicted for serine, threonine and tyrosine amino acids.

2.4. Prediction of putative N-glycosylation and phosphorylation positions

The putative N-glycosylation and phosphorylation sites of SARS-CoV-2 N protein were predicted employing two online servers, one of which was NetNGlyc 1.0 online server used for N-glycosylation positions (Gupta and Brunak 2004) (http://www.cbs.dtu.dk) and the other of which was NetPhos 3.1 online server used for the putative phosphorylation positions (Blom et al., 2004) (http://www.cbs.dtu.dk). The putative phosphorylation positions were predicted for serine, threonine and tyrosine amino acids.

2.5. Prediction and comparative analysis of 3D structure of the N protein

The N protein sequences of SARS-CoV-2 samples from Wuhan/China (YP_009724397.2) and Shanghai/China (QHD43423.2) were used for the generation of predicted 3D structures using Phyre^2 (Protein homology/analogy recognition https://zhanglab.ccmb.med.umich.edu) (Zhang & Skolnick 2004).

3. Results and discussion

3.1. Phylogeny and relative search for the N protein

The nucleocapsid protein (N protein) sequences were retrieved and compared using nucleotide mega BLAST for the estimations of the similarity levels existed in the taxonomically related CoV N proteins. The results of nucleotide blast search were shown in Table 1.

According to the results, the highest coverage and identity values were calculated between the SARS-CoV-2 samples from Kayseri/Turkey, Shanghai/China, Risaralda/ Colombia and Bat-CoV RaTG13 (100/99.05) whereas the lowest coverage and identity values were found to be between most of SARS-CoV-2 samples and Camel CoV (74/37.16). By the relevance of these data, the most similar N protein nucleotide sequence to SARS-CoV-2 N protein nucleotide sequence was found in Bat-CoV (Bat-CoV RaTG13) among all of the taxonomically related CoVs, in agreement with previous results (Cui et al., 2019; Zhang et al., 2020).

3.2. Phylogenetic analyzes

Phylogeny constructed based on the SARS-CoV-2 N protein was tested with nucleotide and protein sequences. Four main groups were identified in both of the nucleotide and protein based joining phylogenetic trees shown in Fig. 1. The main groups consist of individuals from Igacovirus (orange), Embecovirus (green), Merbecovirus (turquoise) and Sarbecovirus (yellow). All the SARS-CoV-2 N protein sequences were grouped in Sarbecovirus group with SARS, bat, pangolin and civet CoVs by 93% and 99% bootstrap values in the nucleotide sequence and protein sequence based phylogenetic trees, respectively. There were two Sarbecovirus subgroups in both phylogenetic trees: named as A and B in the nucleotide sequence tree, and C and D in the protein based phylogenetic tree. The A and C subgroups included bat, SARS and civet CoVs while the B and D subgroups consisted of SARS-CoV-2, and bat and pangolin CoVs. In consistency with the previous studies, pangolin CoV, Bat-CoV RATG13, BAT-SL ZC45 and BAT-SL ZXC21 from bat CoVs grouped with SARS-CoV-2 in both trees (Chen et al., 2020; Tilocca et al., 2020; Wu et al., 2020; Zhang et al., 2020). Additionally, pangolin CoVs (the nucleotide and protein sequences of GX-5PE, GX-2PV, GX-5PL, GX-P1E, GX-P4L and the nucleotide sequence from

Table 1
Nucleotide blast results of N proteins of novel SARS-CoV-2 and taxonomically related CoVs. Cover/Identity values shown in percentage.

	Taxonomically related CoVs					
SARS-CoV-2 Sample Country/City	**Bat-CoV RaTG13**	**Pangolin-CoV**	**SARS-CoV BJ01**	**Civet-CoV**	**MERS-CoV**	**Camel-CoV**
Wuhan/China	100/96.90	100/96.19	100/90.52	100/90.05	84/48.39	74/37.16
Shanghai/China	100/99.05	100/97.85	100/90.52	100/90.05	84/48.39	74/37.16
Richmond/USA	100/98.57	100/97.37	100/90.05	100/89.57	84/48.39	74/37.16
Kanagawa/Japan	100/98.81	100/97.61	100/90.28	100/89.81	84/48.39	74/37.16
Tokyo/Japan	100/98.81	100/97.61	100/90.28	100/89.81	84/48.12	74/37.46
Alexandroupolis/Greece	100/98.33	100/97.14	100/90.05	100/89.57	84/48.39	74/37.16
Risaralda/Colombia	100/99.05	100/97.85	100/90.52	100/90.05	84/48.39	74/37.16
Valencia/Spain	100/98.81	100/97.85	100/90.28	100/89.81	84/48.39	74/37.16
Kayseri/Turkey	100/99.05	100/97.85	100/90.52	100/90.05	84/48.39	74/37.16

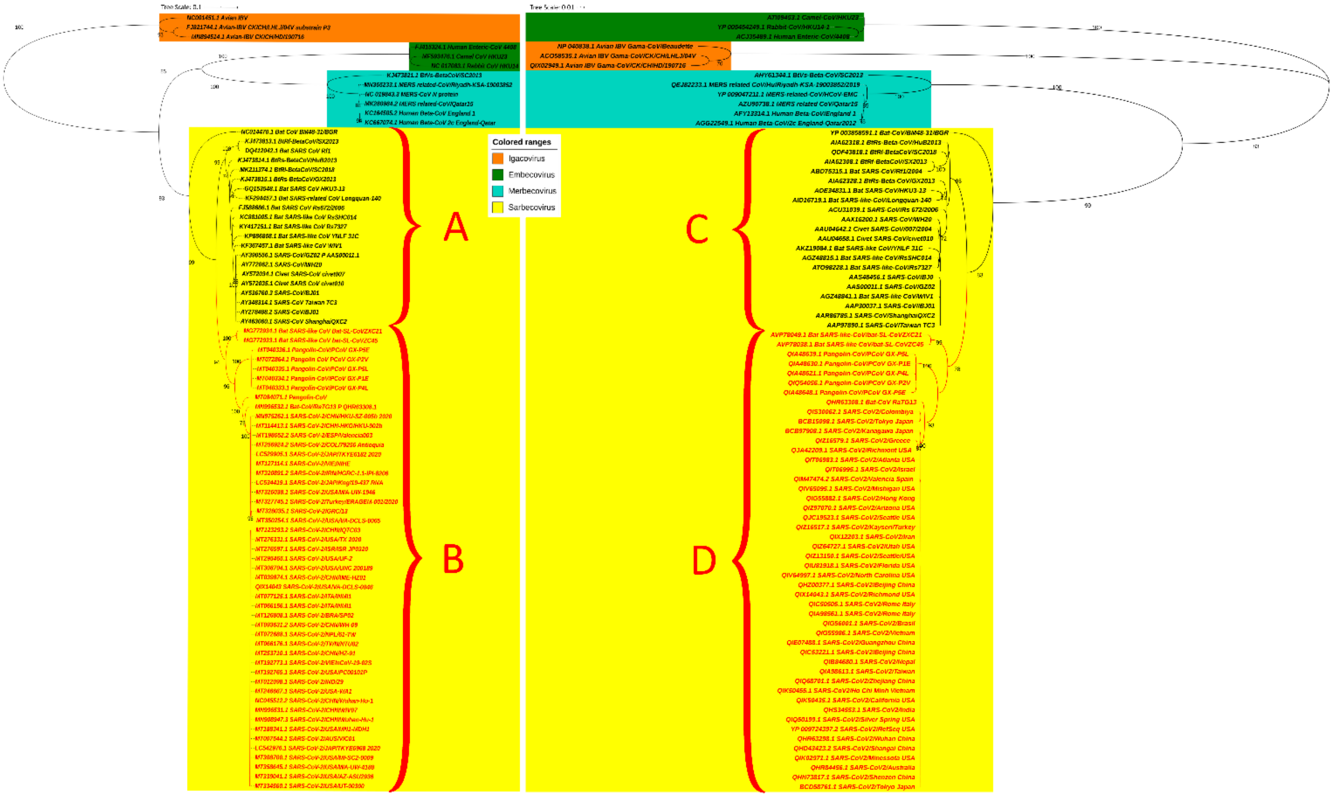


Fig. 1. Nucleotide blast results of N proteins of novel SARS-CoV-2 and taxonomically related CoVs. Cover/Identity values shown in percentage.

Fig. 2. ML phylogenetic tree of SARS-CoV-2 based on amino acid sequences of the N protein.

viral genome MT084071.1) were also grouped with SARS-CoV-2 in subgroup B for the N proteins.

Protein based phylogenetic tree of SARS-CoV-2 was shown in Fig. 2. According to the results, among 40 SARS-CoV-2 sequences, Tokyo/Japan (BCB15098), Alexandroupolis/Greece (QIZ16579), Richmont/USA (QJA42209), Kanagawa/Japan (BCB97908), Valencia/Spain (QIM47474) and Bogota/Colombia (QIS30062) were diverged from the others. Especially, the Alexandroupolis/Greece (QIZ16579) and Richmont/USA (QJA42209) samples were clustered together forming a small group with 86% bootstrap value. Also there were a SARS-CoV-2 sequence from Richmont/USA (QIX14043.1) and nine other sequences from USA, Richmont/USA (QJA42209) diverged from the rest of USA samples.

3.3. Domain, sequence variation analysis and amino acid substitutions

The amino acid sequences of SARS-CoV-2 N proteins were aligned with some the other members of Sarbecovirus to reveal sequence variation and amino acid substitutions. The results of alignment were shown in Fig. 3. The results of domain analyzes conducted using Pfam and HAMAP databases showed that the sequence between 14 and 377 positions displayed matches with family Corona_nucleocora domain in Pfam database and the two sequences displayed matches in HAMAP database (the first match in the sequence was between 41 and 186 on N terminal with CoV RNA-binding domain and the second match in the sequence was between 258 and 361 on C terminal with N protein dimerization domain). Serine x”on position 176 was phosphorylated. Tajima’s D, segregating sites and nucleotide diversities (π) were calculated for the selected 19 Sarbecovirus members and they were also used for sequence variation analyzes as -0.337920, 0.053546 and 86, respectively.

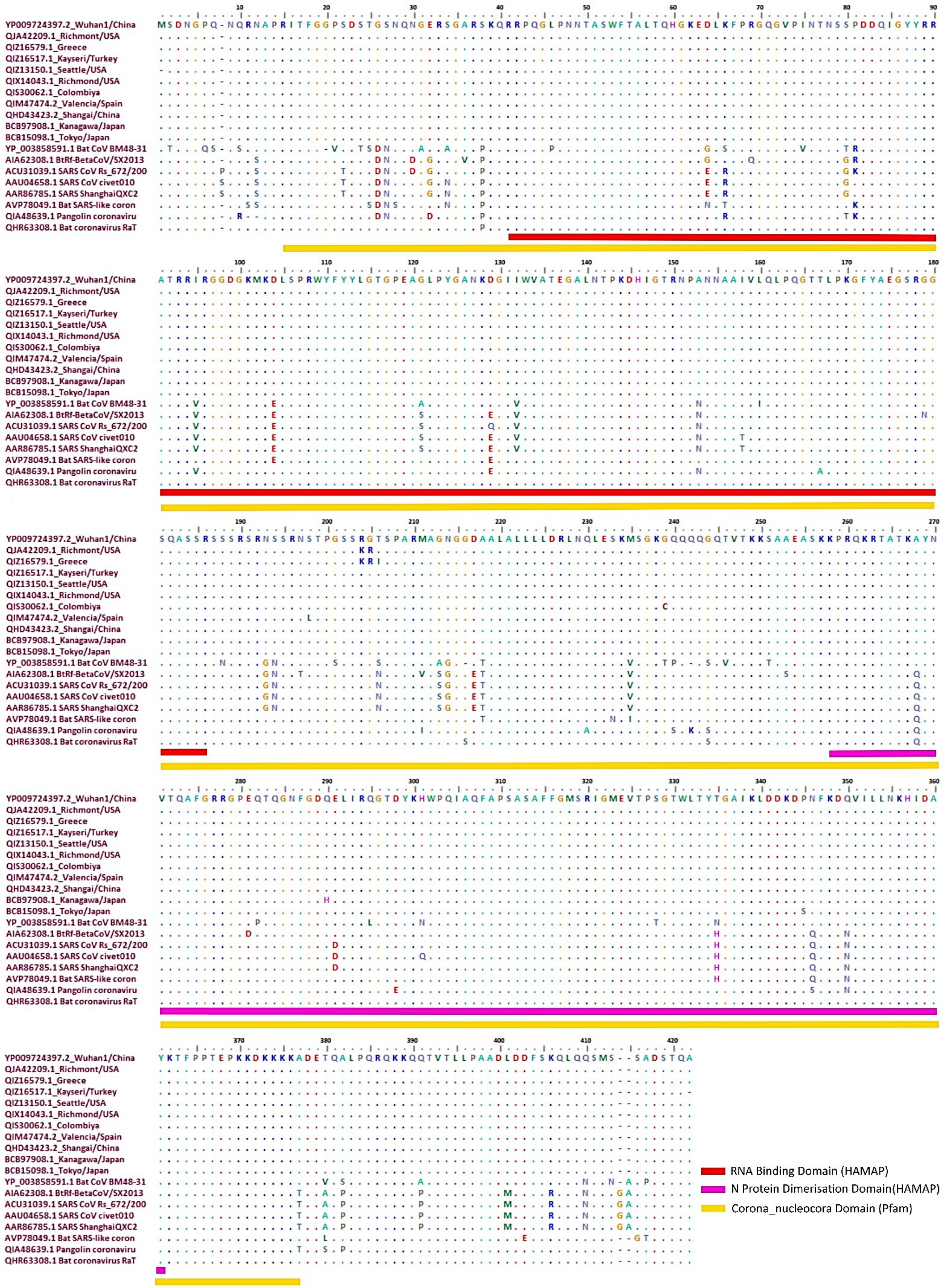


Fig. 3. The amino acid sequences of SARS-CoV-2 and the other members of Sarbecovirus N proteins based on multiple sequence alignment.

Table 2
Amino Acid Substitutions of SARS-CoV-2s.

Substitution	Position	Amino Acid Present	Amino Acid Replaced	SARS-CoV-2
S198L	198	Serin (S)	Leucine (L)	Valencia/Spain (QIM47474.2)
R204K	204	Arginine (R)	Lysine (K)	Richmont/USA (QJA42209.1), Greece (QIZ16579.1)
G205R	205	Glycine (G)	Arginine (R)	Richmont/USA (QJA42209.1), Greece (QIZ16579.1)
T206I	206	Threonine (T)	Isoleucine (I)	Greece (QIZ16579.1)
G239H	239	Glycine (G)	Cysteine (C)	Colombia (QIB84680.1)
Q290H	290	Glutamine (Q)	Histidine (H)	Kanagawa/Japan (BCB97908.1)
P345S	345	Proline (P)	Serin (S)	Tokyo/Japan (BCB15098.1)

According to the sequence variation analysis, there were some amino acid substitutions in both the samples of SARS-CoV-2, and between SARS-CoV-2 and the other Sarbecoviruses. The substitutions among the samples of SARS-CoV-2 were shown in Table 2 and Fig. 3. The substitutions detected were as following: serine (S) replaced with leucine (L) in position 198 (S198L, Valencia/Spain - QIM47474.2); arginine (R) replaced with lysine (K) in position 204; glycine (G) replaced with arginine (R) in position 205 (R204K and G204R, Richmont/USA - QJA42209.1 and Greece - QIZ16579.1), Threonine (T) replaced with Isoleucine (I) in position 206 (T206I, Greece - QIZ16579.1); glycine (G) replaced with cysteine (C) in position 239 (G239H, Colombia - QIB84680.1); glutamine (Q) replaced with histidine (H) in position 290 (Q290H, Kanagawa/Japan - BCB97908.1); proline (P) replaced with serin (S) in position 345 (P345S, Tokyo/Japan - BCB15098.1). Among the amino acid substitutions, Q290H and P345S substitutions were occurred at N protein dimerization domain while other substitutions were occurred between RNA binding and dimerization domains. SARS-CoV-2 N proteins from Richmont/USA (QJA42209.1) and Greece (QIZ16579.1) were shown higher substitution numbers, 2 and 3, respectively.

Amino acid substitutions can alter and/or inhibit protein function (Ng & Henikoff, 2006; Teng et al., 2010). There are some studies showing the effects of amino acid substitutions in viruses (Schrauwen et al., 2016; Perera et al., 2019). Thus, the further investigations must be done for the detection of the effects of substitutions occurred in the N protein or other functional and/or structural proteins of SARS-CoV-2.

Total of 86 substitutions were detected between SARS-CoV-2 and the other selected members of Sarbecovirus shown in Fig. 3. Only 29 of these substitutions (33.7%) were detected at the RNA binding and dimerization domains. Some noticeable substitutions detected were as N12S, G25D, S26N, S38P, I95V, D104E, G212S, D129E, I132V, A153N, N193G, S194N, N210G, A217T, M235V, A268Q, T334H, N346Q, Q350N, A377T, A382P and Q410N. Also, some substitutions detected in the different studies and this study were as G25D, S26N, D103E, A217T and T334H substitutions (Wu et al., 2020), A267Q (Cagliani et al., 2020), and structurally relevant amino acid substitutions T380A and Q410N (Ceraolo & Giorgi, 2020).

3.4. N-glycosylation and phosphorylation positions of SARS-CoV-2 N protein

Phosphorylation is one of the important post-transcriptional modifications of viral proteins. Phosphorylation regulates vital processes, including replication, transcription, RNA binding and viral assembly (Huang et al., 2015). Although it is not known exactly how N proteins fulfill their functions, it is estimated that the phosphorylated N protein serves as unifying of the RNA genome into the virion and forming replication - transcription complex (Carlson et al., 2020). N-glycosylation affects viral protein functions by altering protein immunogenicity and facilitating viral protein interactions with host receptors (Mossenta et al., 2017). Therefore, the detection of putative N-glycosylation and phosphorylation positions may provide useful information for future studies especially related with vaccine and/or antiviral drug development. Two online servers used for the detection of putative N-glycosylation and phosphorylation positions were NetNGlyc 1.0 and NetPhos 3.1 servers. The results of the predictions were shown in Fig. 4.

The possible N-glycosylation positions of SARS-CoV-2 N protein were predicted as 47 NNTA (68% possibility) and 269 NVTQ (82% possibility) using NetNGlyc 1.0 server, in consistency with the results of Supekar et al., (2020). The two positions for N-glycosylation confirmed by the authors were with lower possibility for 47 NNTA (53%) and higher possibility for 269 NVTQ (94%) (Supekar et al. 2020). The N-glycosylation positions for 269 NVTQ and 47 NNTA were in the dimerization domain and RNA binding domain at the beginning, respectively. Additionally, three more N-glycosylation positions were also predicted with low possibility at 77 NSSP (21%), 192 NSSR (45%) and 196 NSTP (13%) positions.

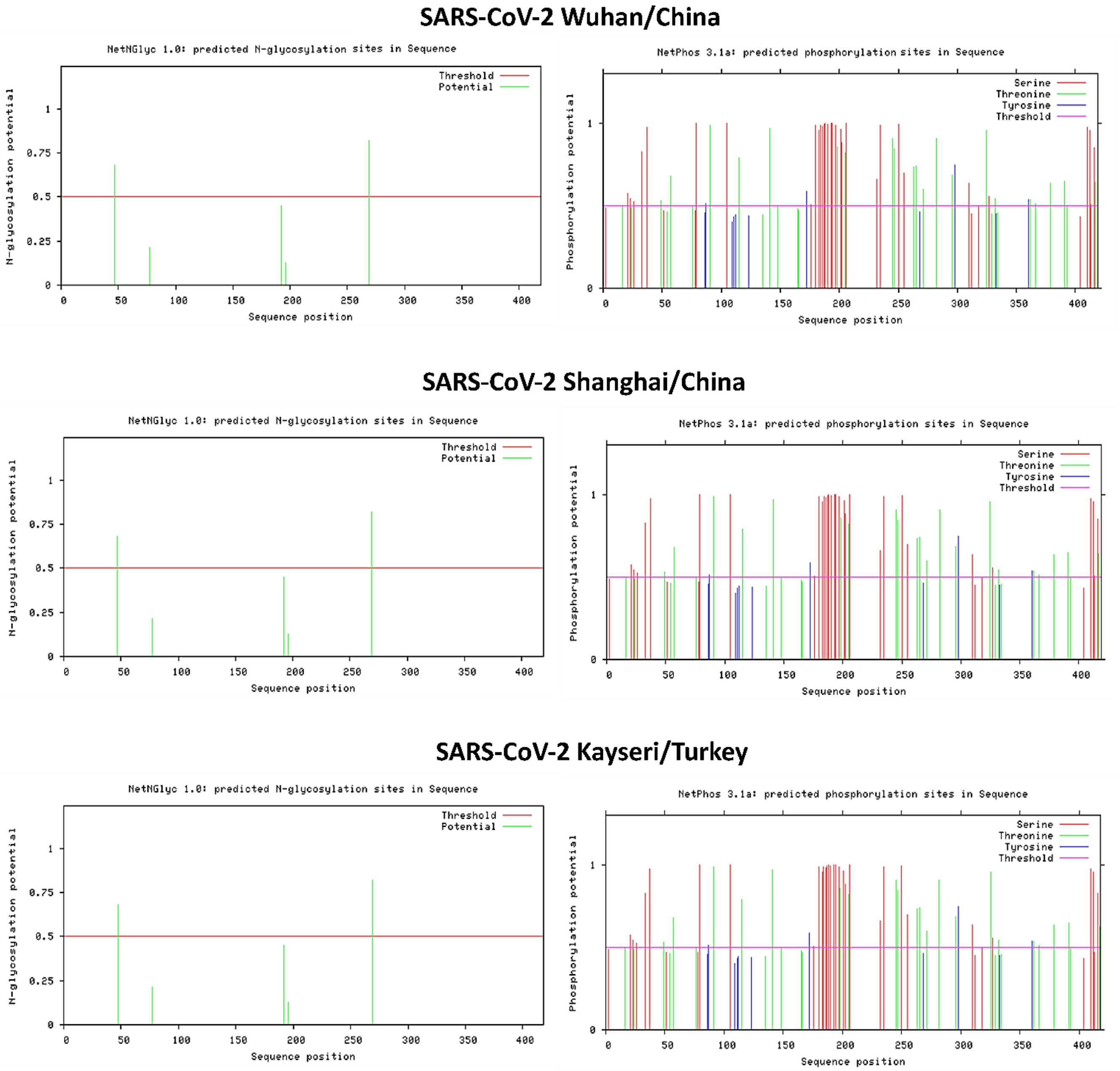
The possible serine, threonine and tyrosine phosphorylation of the N protein was predicted to be at the 100 position (with 50% possibility threshold) for all the SARS-CoV-2 samples. The extensive analysis of SARS-CoV-2 sample from Kayseri/Turkey revealed that the distribution of possibilities occurred as following: 41 at 50-60%, 14 at 60-70%, 11 at 70-80%, 10 at 80-90% and 24 at 90-100%.

3.5. Comparative analysis of 3D structure of CoV N protein

The analysis of the 3D structures of the N proteins gives us information for understanding of viral packaging, taxonomical relatedness and how it functions. For that, the putative 3D structures of N proteins from the two SARS-CoV-2 samples and 11 taxonomically related previous CoVs were generated using Phyre^2 server and the assessments of topological similarities of the 3D structures of the N proteins were done by analyzing TM Scores. The results of the assessments were given in Table 3.

Xu and Zhang (2010) stated that protein pairs with a TM score >0.5 are usually not in the same fold and if TM score falls below 0.17, the protein pairs are assumed not in the same fold.

In this study, the highest TM score was detected between Shanghai/China SARS-CoV-2 and Beta-CoV SX2013 (0.4067)


SARS-CoV-2 Wuhan/China

SARS-CoV-2 Shanghai/China

SARS-CoV-2 Kayseri/Turkey

Fig. 4. The results of predicted putative N-glycosylation and phosphorylation positions on three SARS-CoV-2 N proteins.

Table 3.
The results of the similarity assessments (TM score) between SARS-CoV-2 and taxonomically related previous CoVs N protein structures.

SARS-CoV-2	Taxonomically Related CoVs					
Sample	**Shanghai/China** (QHD43423.2)	**Bat-COV RaTG13** (QHR63308.1)	**Pangolin-CoV** (QIA48639.1)	**SARS-CoV BJ01** (AAR86785.1)	**Civet-CoV** (AAU04642.1)	**MERS-CoV** (YP009047211.1)
Wuhan/China (YP_009724397.2)	0.3946	0.3680	0.2513	0.3894	0.2904	0.2223
Shanghai/China (QHD43423.2)	1	0.3987	0.2953	0.3083	0.3246	0.1788

SARS-CoV-2	Taxonomically related CoVs					
Sample	**Camel-CoV** (ATI09453.1)	**Beta-CoV SX2013** (AIA62308.1)	**SARS-CoV** (ACU31039.1)	**Avian IBV** (NP040838.1)	**Human Enteric-CoV** (ACJ35489.1)	**Human Beta-CoV** (AFY13314.1)
Wuhan/China (YP_009724397.2)	0.1681	0.3955	0.3565	0.1726	0.1637	0.1690
Shanghai/China (QHD43423.2)	0.1558	0.4067	0.3050	0.1644	0.1569	0.2069

whereas the lowest was detected between Wuhan/China SARS-CoV-2 and Camel CoV (0.1558). The TM scores between SARS-CoV-2 samples and Camel-CoV, Human Enteric CoV, Human Beta-CoV, Avian IBV and MERS-CoV samples were calculated as equal or below 1.7. The TM score between SARS-CoV samples and Pangolin-CoV/Civet-CoV was resulted as higher. The average TM scores between SARS-CoV-2, and Beta-CoV SX2013, Bat-COV RaTG13, SARS-CoV BJ01and SARS-CoV were resulted as 0.3660 but not exceed 0.5. Interestingly, The TM score between Wuhan/China and Shanghai/China SARS-CoV-2 samples was resulted as 0.3946. In the light of this data, although the TM scores between SARS-CoV-2 and other taxonomically related CoVs were shown consistency with the taxonomical data, the 3D structures of the selected CoV samples were not at the same fold. Also, it can be said that the 3D structures of SARS-CoV-2 N proteins were shown significant divergences.

4. Conclusion

The novel coronavirus SARS-CoV-2, the cause of Covid 19 infection, created one of the biggest outbreak in the world history. SARS-CoV-2 has structural proteins, including S, E, M and N proteins. N protein involves in viral assembly, replication, transcription and translation (Chen et al., 2020). The sequence analysis of SARS-CoV-2 N protein RaTG13 using cover and identity values revealed that the most similar N protein sequence to SARS-CoV-2 belongs to Bat-CoV. However, the lowest cover and identity values were detected for Camel CoV. The phylogenetic analysis of SARS-CoV-2 was conducted using both of the nucleotide and protein sequences of the N protein. Four main (Igacovirus, Embecovirus, Merbecovirus and Sarbecovirus) groups identified in both nucleotide and protein trees and SARS-CoV-2 were placed in Sarbecovirus with SARS-CoV, and bat, pangolin and civet CoVs. Bat-CoV RATG13, BAT-SL ZC45 and BAT-SL ZXC21 were grouped with SARS-CoV-2 in both trees in agreement with the related literature. Additionally, a third ML tree was constructed based on the protein sequences of 40 SARS-CoV-2 N protein samples. By the phylogenetic analysis, it was shown that some of the SARS-CoV-2 N protein samples showed divergences from the other selected samples. The diverged SARS-CoV-2 N protein samples were Tokyo/Japan (BCB15098), Alexandroupolis/Greece (QIZ16579), Richmont/USA (QJA42209), Kanagawa/Japan (BCB97908), Valencia/Spain (QIM47474) and Bogota/Colombia (QIS30062).

The domain search was conducted using of both Pfam and HAMAP databases. Three domains were detected as Corona_nucleocora (Pfam), N terminal CoV RNA-binding domain (HAMAP) and C terminal N protein dimerization domain (HAMAP). The sequence variation analysis revealed seven amino acid substations within the selected SARS-CoV-2 samples. The Richmont/USA (QJA42209.1) and Greece (QIZ16579.1) SARS-CoV-2 samples were shown as having higher substitution numbers, 2 and 3 substitutions, respectively. The possible N-glycosylation positions of SARS-CoV-2 N protein were predicted as 47 NNTA with 68% and 269 NVTQ with 82%. The possible serine, threonine and tyrosine phosphorylations were predicted for 100 positions with above 50% possibility (34 of them were having higher than 80% possibility). The TM score analysis revealed that the 3D structures of SARS-CoV-2 N protein and taxonomically related CoVs were not at the same fold. Also, the TM score of N protein pairs of SARS-CoV-2 samples was calculated as 0.3946. In the light of this data, although the analysis of 3D structure data was shown to have consistency with the taxonomy of the coronavirus, SARS-CoV-2 and taxonomically related CoV N proteins, they showed significant divergences.

In last three decades, the world has encountered with some severe outbreaks caused by CoV family, including SARS (2002/03), MERS (2012/15) and Covid19 (2019-still continues). The developments of vaccines and therapeutics for fighting with the current and potential future outbreaks are crucial. Analysis, identification and interpretation of all components of pathogenic CoVs will contribute in fighting with the disease. In hope, the information gained in this study will make contributions in fighting with the current and future CoV outbreaks.

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Supplementary

Suppl. Table 1. NCBI GenBank accession numbers and some features of retrieved SARS-CoV-2 sequences.

Seq No	Protein Accession Number	Source Viral Genome Accession Num.	Nucleotide Position in The Genome	City/Country
1	YP_009724397.2	NC_045512.2	28274-29533	Wuhan/China
2	QHR63298.1	MN996531.1	28261-29520	Wuhan/China
3	QHZ00377.1	MT039874.1	28262-29521	Beijing/China
4	QIC53221.1	MT093631.2	28261-29520	Beijing/China
5	QHD43423.2	MN908947.3	28274-29533	Shanghai/China
6	QIQ68701.1	MT253710.1	28220-29479	Zhejiang/China
7	QHN73817.1	MN975262.1	28274-29533	Shenzhen/China
8	QIE07488.1	MT123293.2	28268-29527	Guangzhou/China
9	QIG55882.1	MT114413.1	28274-29533	Hong Kong/China
10	QIA98613.1	MT066176.1	28274-29533	Taipei/Taiwan
11	QIX14043.1	MT322424.1	28274-29533	Richmond/USA
12	QIZ64727.1	MT334560.1	28235-29494	Utah/USA
13	QJC19523.1	MT358645.1	28243-29502	Seattle/USA
14	QIV65095.1	MT308700.1	28225-29484	Michigan/USA
15	QIQ50199.1	MT246667.1	28270-29529	Maryland/USA
16	QJA42209.1	MT350254.1	28273-29532	Richmond/USA
17	QIU81918.1	MT295465.1	28264-29523	Florida/USA
18	QIK50435.1	MT192765.1	28267-29526	California/USA
19	QIV64997.1	MT308704.1	28249-29508	North Carolina/USA
20	QIK02971.1	MT188341.1	28223-29482	Minnesota/USA
21	QIT06983.1	MT276331.1	28274-29533	Atlanta/USA
22	QIZ97070.1	MT339041.1	28274-29533	Arizona/USA
23	QIZ13150.1	MT326038.1	27903-29162	Seattle/USA
24	BCB97908.1	LC534419.1	28262-29521	Kanagawa/Japan
25	BCB15098.1	LC529905.1	28274-29533	Tokyo/Japan
26	BCD58761.1	LC542976.1	28274-29533	Tokyo/Japan
27	QIG55986.1	MT127114.1	143-1402	Hanoi/Vietnam
28	QIK50455.1	MT192773.1	28273-29532	Ho Chi Minh/Vietnam
29	QIC50505.1	MT077125.1	28218-29476	Rome/Italy
30	QIA98561.1	MT066156.1	28274-29533	Rome/Italy
31	QIT06995.1	MT276597.1	28254-29513	Ness Ziona/Israel
32	QIS30062.1	MT256924.2	28220-29479	Bogota/Colombia
33	QIB84680.1	MT072688.1	28259-29518	Risaralda/Colombia
34	QIZ16579.1	MT328035.1	28274-29533	Alexandropoulos/Greece
35	QIG56001.1	MT126808.1	28274-29533	Sao Paulo/Brazil
36	QIM47474.2	MT198652.2	28220-29479	Valencia/Spain
37	QHS34553.1	MT012098.1	28258-29517	Maharashtra/India
38	QHR84456.1	MT007544.1	28274-29533	Melbourne/Australia
39	QIX12203.1	MT320891.2	28230-29489	Tehran/Iran
Protein length	**419 aa**	**Nucleotide length**	**1260 bp**	

Suppl. Table 2. NCBI GenBank accession numbers and some features of retrieved other CoV sequences.

Seq No	Source	Protein Accession Number	Protein length	Source Viral Genome Accession Num.	Nucleotide length	Nucleotide Position in The Genome
1	Bat-CoV RatG13	QHR63308.1	419	MN996532.1	1260	28240-29499
2	Bat-CoV WIV1	AGZ48841.1	422	KF367457.1	1269	28686-29954
3	Bat-CoV HKU3-13	ADE34831.1	421	GQ153548.1	1266	28073-29338
4	Bat-CoV RsSHC014	AGZ48815.1	422	KC881005.1	1269	28162-29430
5	Bat-CoV YNLF_31C	AKZ19084.1	421	KP886808.1	1266	28103-29368
6	Bat-CoV Rf1/2004	ABD75315.1	421	DQ412042.1	1266	28084-29349
7	Bat-CoV bat-SL-CoVZXC21	AVP78049.1	419	MG772934.1	1260	28110-29369
8	Bat-CoV Longquan-140	AID16719.1	421	KF294457.1	1266	28072-29337
9	Bat-CoV Rs7327	ATO98228.1	422	KY417151.1	1269	28684-29952
10	Bat-CoV bat-SL-CoVZC45	AVP78038.1	419	MG772933.1	1260	28179-29438
11	Bat BtRs-Beta-CoV/HuB2013	AIA62318.1	420	KJ473814.1	1263	28049-29311
12	Bat BtRs-Beta-CoV/GX2013	AIA62328.1	422	KJ473815.1	1269	27865-29133
13	Bat BtRf-Beta-CoV/SX2013	AIA62308.1	421	KJ473813.1	1266	27849-29114
14	Bat BtRl-Beta-CoV/SC2018	QDF43818.1	421	MK211374.1	1266	28076-29341
15	Bat-CoV BM48-31/BGR/2008	YP_003858591.1	417	NC_014470.1	1254	27665-28918
16	Bat –Beta-CoV/SC2013	AHY61344.1	434	KJ473821.1	1305	28819-30123
17	Pangolin-CoV isolate MP789	----	---	MT084071.1	1260	25752-27213
18	Pangolin-CoV PCoV_GX-P5E	QIA48648.1	417	MT040336.1	1254	28226-29479
19	Pangolin-CoV PCoV_GX-P5L	QIA48639.1	417	MT040335.1	1254	28230-29483
20	Pangolin-CoV PCoV_GX-P1E	QIA48630.1	417	MT040334.1	1254	28224-29477
21	Pangolin-CoV PCoV_GX-P4L	QIA48621.1	417	MT040333.1	1254	28229-29482
22	Pangolin-CoV_GX-P2V	QIQ54056.1	417	MT072864.1	1254	28218-29471
23	Civet-CoV 007/2004	AAU04642.1	422	AY572034.1	1269	28123-29391
24	Civet-CoV civet010	AAU04658.1	422	AY572035.1	1269	28101-29369
25	Rabbit-CoV HKU14	YP_005454249.1	444	NC_017083.1	1335	29462-30796
26	Avian IBV Gama-CoV Beaudette	NP_040838.1	409	NC_001451.1	1230	25873-27102
27	Avian IBV Gama-CoV CK/CH/LHLJ/04V	ACO58535.1	409	FJ821744.1	1230	1-1230
28	Avian IBV Gama-CoV	QIX02949.1	409	MN894514.1	1230	1-1230
29	Camel-CoV HKU23	ATI09453.1	448	MF593476.1	1346	29227-30573
30	Human Beta-CoV England 1	AFY13314.1	411	KC164505.2	1236	28565-29800
31	Human Beta-CoV 2c England-Qatar/2012	AGG22549.1	411	KC667074.1	1236	28566-29801
32	Human Enteric-CoV 4408	ACJ35489.1	449	FJ415324.1	1347	29394-30740
33	Human MERS-CoV HCoV-EMC	YP_009047211.1	413	NC_019843.3	1242	28566-29807
34	HCoV MERS Riyadh-KSA-19003852/2019	QEJ82233.1	413	MN365233.1	1242	28566-29807
35	MERS related-CoV Qatar15	AZU90738.1	413	MK280984.2	1242	28539-29780
36	Human SARS-CoV WH20	AAX16200.1	422	AY772062.1	1269	27853-29121
37	Human SARS-CoV GZ02	AAS00011.1	422	AY390556.1	1269	28149-29417
38	Human SARS-CoV BJ01	AAS48456.1	422	AY536760.3	1269	81-1349
39	Human SARS-CoV BJ01	AAP30037.1	422	AY278488.2	1269	28101-29369
40	Human SARS-CoV Rs_672/2006	ACU31039.1	422	FJ588686.1	1269	27520-28788
41	Human SARS-CoV ShanghaiQXC2	AAR86785.1	422	AY463060.1	1269	27462-28730
42	Human SARS-CoV Taiwan TC3	AAP97890.1	422	AY348314.1	1269	28051-29319

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Research article

A new species from Turkey: *Eleocharis divaricata* (Cyperaceae) and a note for *E. atropurpurea*

Mustafa Keskin[1], David Merrick[2]

[1] *Marmara University, Institute of Pure and Applied Sciences, Biology Program, 34722, Goztepe, Istanbul, Turkey*
[2] *Durham University, Department of Mathematical Sciences, Durham, DH1 3LE, United Kingdom*

Abstract

In this study, a new species, *Eleocharis divaricata*, is introduced to science. It is easily separated from other species by its short and divaricate stems, blackish-purple achenes, and amphicarpic proliferation. Also, the presence of *E. atropurpurea* in Turkey is discussed. Finally, a new identification key is proposed for all *Eleocharis* species in Turkey.

Keywords: *Eleocharis atropurpurea; E. divaricata; new species; sedge family; Turkey*

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1. Introduction

The sedge family, or *Cyperaceae*, is the third-largest monocot family, consisting of an estimated 5.000 species in 104 genera. The largest genera with approximate numbers of species are *Carex*, 2000 sp., *Cyperus*, 550 sp. and *Eleocharis*, 200 sp. (Goetghebeur, 1998; Simpson and Inglis, 2001).

The genus *Eleocharis* is characterized by the leafless stem, achene type, and toothed perianth bristles.

Almost all species of *Eleocharis* are quite similar to the untrained eye. Further, their natural habitats are similar, with a preference for creekside, swampy, semi-marshy areas, or ponds.

In Turkey, the genus is represented by seven species (*E. quinqueflora* (Hartmann) O. Schwarz, *E. acicularis* (L.) Roemer & Schultes, *E. atropurpurea* (Retz.) C. Presl, *E. palustris* (L.) Roemer & Schultes, *E. mitracarpa* Steudel, *E. uniglumis* (Link) Schultes and *E. carniolica* W.D.J. Koch) according to Flora of Turkey (Tan, 1985).

2. Materials and methods

On a social networking site an interesting specimen was shared as *E. atropurpurea* with detailed photographs by the second author who is a citizen scholar, but this identification was doubted by the first author who is authoring the genus *Eleocharis* for Turkey's Flora.

Later, samples were collected and subjected to scientific examination, leading to a conclusion that, they are not *E. atropurpurea*, and they belong to a new species close to *E. atropurpurea* (Retz.) C. Presl, and *E. geniculata* (L.) Roem. & Schult.

3. Results

3.1. The genus Eleocharis in Turkey

3.1.1. A new species for science from Turkey

Eleocharis divaricata **M.Keskin sp. nov.** (Fig. 1).

Holotype: Turkey, Antalya: Konyaaltı, Boğa çayı vicinity, 4.xii.2020, D.Merrick (Holo NGBB)

Diagnose: It is related to *E. atropurpurea* (Retz.) C. Presl and *E. geniculata* (L.) Roem. & Schult. It is easily separated from them by its achenes having a greenish stylopodium, amphi-

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* Corresponding author.
E-mail address: trifolium@hotmail.com (M. Keskin).

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Table
A comparison of *E. atropurpurea*, *E. geniculata* and *E. divaricata* (***=not any knowledge)

	E. atropurpurea		***E. geniculata***		***E. divaricata***
	Blake, 1939	Svenson, 1929	Cosewic, 2009	Menapace, 2020	in this study
Stem	filiform, at most 0.3 mm wide	filiform, strict or bent	thin or filiform, c.1 mm at base; 0.5-0.6 mm wide at the tip	0.2-1 mm	thin or filiform, caespitose, c.1 mm at base; 0.5-0.6 mm wide at tip; amphicarpic proliferation
Glumes	1-1.3 mm	***	1.5 - 2 mm	0.8-3 mm, ovate to elliptic	1.7-2.0 mm
Stamen	1	1-3	***	***	2
Achenes	0.6-0.65	0.5 mm	0.7–1.1 mm	brown ripening to black, biconvex, orbicular to obpyriform, 0.5–1.1	1 mm, blackish purple, stipitate, orbicular to obpyriform
Stylopodium	very short, depressed, white	markedly flattened, or absent	with a flat and fairly wide tubercle, green	stramineous to whitish, umbonate to subconic, 0.2–0.4 × 0.2–0.5 mm, apex rounded to acute	deltoid narrow than wide of achenes, usually light green
Bristles	4-6, thin, white, transparent, shortly spinose or smooth; shorter than achenes or sometimes absent; shorter than achenes or equal	thin, transparent, usually stubby or wanting; shorter than achenes or equal	thin, purplish-brown, equal to achenes	(0–)4–8, typically 7, red-brown, rarely whitish, vestigial to much-exceeding tubercle, typically equaling achene,	(6-)7, white, spinose at upper parts or completely, longer than achenes

carpic proliferation, long and wide glumes, and white perianth bristles longer than the achenes (Table).

Description: Plants annual or short-lived perennial with amphicarpic proliferation. Stems 7-10 cm, thin or filiform, caespitose, c.1 mm at base, 0.5-0.6 mm wide at the tip, shiny dark green.

Leaf sheaths 1 or 2, reddish or mauve, obliquely truncate. Inflorescences 4-8 mm ovate-deltoid, obtuse at apex; many-flowered; flowers densely imbricate.

Lower glumes 2, equal and sterile, large green carina on the back, 3-nerved and longer than others, each glume covering up to half the spikelets. Glumes 1.7-2.0 x 1.1-1.3 mm, median nerve green and carinate, brown outward toward the edge, outer parts transparent, membranous, obscurely veined.

Stamens 2; filaments longer than achenes and bristles, anthers 0.8-1.1 mm. Stigma 2, hairy. Perianth bristles (6-)7, white, spinose on the upper parts or completely, longer than achenes.

Achenes obpyriform; thickly bordered at the edge, 1 mm long, widest at the middle, striate, blackish-purple, stipitate at the base; stylopodium deltoid narrower than the achene, usually light green or sometimes whitish.

3.2. A note on the presence of E. atropurpurea in Turkey

E. atropurpurea is known from a single gathering in Turkey (Tan, 1985).:

Muğla: Köyceğiz, Dalyan, Sülüngür Lake, Uotila 30598!

The species, widespread in the tropics and subtropics, is introduced in Europe, and the specimen in Edinburgh Herbarium was investigated by the first author.

Consequentially, the single specimen from Turkey is certainly not *E. atropurpurea* due to its general structure being long-rhizomatous, perennial, long and thick-stemmed, and the achenes not being black.

It is equal to abnormal form of *E. palustris* (L.) Roemer & Schultes with small inflorescence that is the one that is very common in Turkey. For this reason, *E. atropurpurea* has been removed from the list of Turkey's Flora (Fig. 2.).

3.3. A new identification key

1. Achenes blackish-purple *E. divaricata*

1. Achenes usually brownish

  2. Stylopodium absent *E. quinqueflora*

  2. Stylopodium present

    3. Achenes tri-faced with longitudinal and transverse veins *E. acicularis*

    3. Achenes two-faced, never longitudinally and transversely veined

      4. Stylopodium as broad as achenes, always broader than long *E. mitracarpa*

      4. Stylopodium at most as wide as, but usually narrower than, the achenes

        5. Bristles 6-8 and longer than achenes *E. carniolica*

        5. Bristles usually 4-6 and at most equal to the achenes

          6. Lowest glume of spikelet sterile, encircling the spikelet base; achenes minutely tuberculate-striate or smooth *E. uniglumis*

          6. Lowest two glumes sterile, each halfencircling the spikelet base; achenes usually smooth *E. palustris*

4. Discussion

While seven *Eleocharis* species were reported in Flora of Turkey (Tan, 1985), *E. atropurpurea* was removed from the list for being misidentified and not actually being present in Turkey.

An interesting specimen of *Eleocharis* was collected from Antalya. It is understood that this collected plant differentiates from all other species of *Eleocharis* by its blackish-purple achenes, long bristles, amphicarpic proliferation, long and wide glumes. For this reason, this specimen is introduced to the scientific world as *E. divaricata*. A new key for Turkish *Eleocharis* has been prepared to identify species in Turkey.

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Fig. 1. *Eleocharis divaricata* M.Keskin sp.nov. (1) Habitus, (2) General view, (3) Close up from the base, (4) A single plant, (5) A spikelet, (6) Achenes. (The arrow indicates amphicarpic shoots, all scale bars 1 mm).

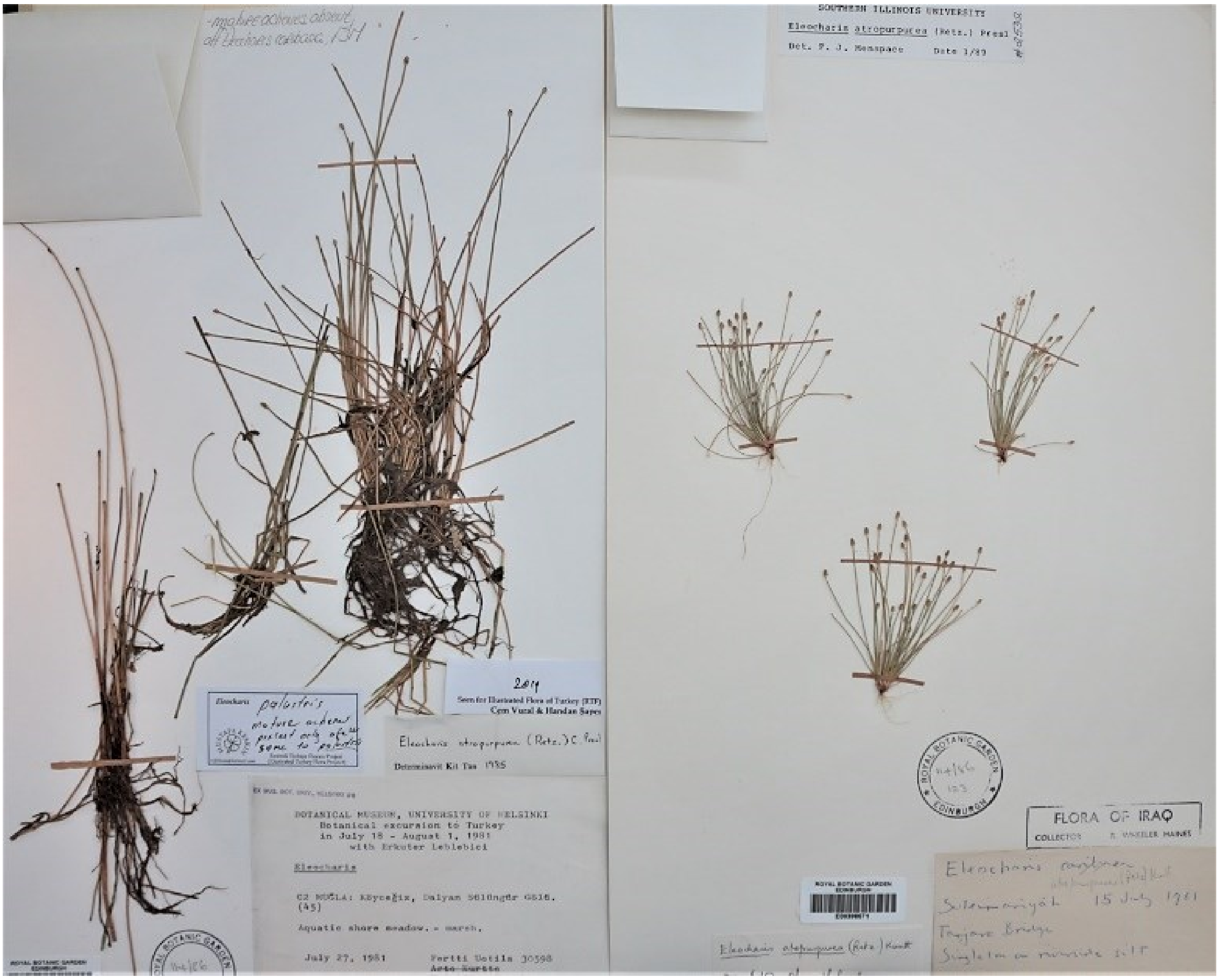

Fig. 2. A visual comparison for (left) the *E. palustris* identified as *E. atropurpurea* in Flora of Turkey, Uotila 30598 and (right) real *E. atropurpurea*.

Conflict of interest: The authors declare that they have no conflict of interests.

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Research article

The effect of biology teaching with concept cartoons based on constructivist learning approach on student achievement and permanence of knowledge

Ali Aslan*[1,2], Tubanur Aslan Engin[3], Gulbubu Kurmanbekova[1], Fethi Kayalar[4], Filiz Kayalar[5], Yalcin Karagoz[6], Adem Engin[3]

[1] *Kyrgyz-Turkish Manas University, Faculty of Science, Department of Biology, 720038, Bishkek, Kyrgyzstan*
[2] *Yuzuncu Yil University, Faculty of Pharmacy, Department of Pharmacology, 65080, Van, Turkey*
[3] *Hakkari University, Vocational School of Health Services, Program of Medical Laboratory Techniques, 30000, Hakkari, Turkey*
[4] *Erzincan Binali Yildirim University, Faculty of Education, Department of Educational Sciences, 24100, Erzincan, Turkey*
[5] *Erzincan Binali Yildirim University, Faculty of Art and Science, Department of Science of Translation, 24100, Erzincan, Turkey*
[6] *Agri Ibrahim Cecen University, Faculty of Pharmacy, Department of Pharmaceutic Botany, 04100, Agri, Turkey*

Abstract

The aim of this study is to investigate the effect of concept cartoons and biology teaching based on a constructivist learning approach on student achievement and permanence of knowledge. The study group consists of 49 students studying in two of the 10th digital classes of Kazım Karabekir Technical and Industrial Vocational High School in the Yunus Emre district of Erzurum. At the stage of data collection, biology achievement tests including three-stage pre-test, post-test and permanence test consisting of 25 questions related to the subject of “Ecosystem Ecology”, which is the third unit of the biology course, were prepared and used from the preparation books of the Student Selection Examination (ÖSS). In the study in which the pretest, posttest control group quasi-experimental design was used, the subjects were applied to the control group with the traditional straight expression method, and the teaching method was applied to the experimental group with the researched concept cartoons. The cartoons were given to the experimental group as a material, and the lesson was presented as slides, supported by cartoons, and actively processed in the form of questions and answers. As a result of the evaluation of the data, there was a statistically significant difference in favor of the experimental group ($p < 0.05$) according to the posttest scores obtained from the achievement test in the experimental group, where the teaching was performed with concept cartoons, and there was a significant difference between the groups in terms of retention test scores made after 4 weeks ($p < 0.05$) was observed. At the end of the study, it was understood that teaching with concept cartoons had a positive effect on providing students to learn biology knowledge compared to the traditional method.

Keywords: *Biology; cartoon; concept cartoons; constructivist teaching method; permanent learning*

1. Introduction

Rapid developments in science and modern technology based on them have been the basis for the development of biology, and have led to an increase and change in knowledge (Buckley and Michel, 2020; Ozyigit, 2020).

These developments made it necessary to make changes in the aims of the education system. With the efforts to prepare science programs in our country since the 1970s in accordance with the developments of the age, and transferring knowledge based on rote learning, education has changed to give students the scientific thinking and behavioral abilities, and methods of

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* Corresponding author.

E-mail address: aliaslan@yyu.edu.tr, ali.aslan@manas.edu.kg (A. Aslan).

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obtaining and using knowledge that they will use throughout their lives (Yesilyurt, 2005). One of the important goals recent education system is to get away from rote-based education and to create permanent knowledge that increases productivity. (Kete et al., 2009; Uslu and Celikoz, 2020).

The emphasis on how to make learning more effective and permanent is the basis of Educational Science. Learning is a process that takes place by organizing the information in short and long-term memory with various methods and applications and making it permanent as a result of interactions with the external environment (Wiewiora et al., 2020). In this process, one of the learning approaches that enable students to actively participate and practice by doing, instead of transferring the information to students' minds sequentially, is the constructivist approach (Inel et al., 2009). This approach to learning means that knowledge cannot be constructed passively or without a personal contribution, that understanding is the result of adaptation, that the person understands the subject by harmonizing with his own experiences, knowledge, and that the knowledge is created as a result of interaction, the language used and the social structure. That plays an important role in interaction (Kwahk and Park, 2018; Langley et al., 2018; Neeley and Leonardi, 2018).

Constructivist approach is the view that is formed by the combination of theories such as productive learning and discovery learning by reconstructing the knowledge by attributing its own meaning as a result of one's experiences and observations. According to this approach, the realization of meaning in interaction with the environment, the stimulus of cognitive contradiction and the search for the nature of what is to be learned are important conditions for learning to occur (Silva and Francis, 2020). Many of the learning psychologists who support the necessity of these conditions agree that learning occurs as a result of the individual's interaction with his environment and creates a change in the individual's behavior. The interaction between the individual and his environment is possible with the effect of the stimulus received by the individual and the individual's reaction to the stimulus (da Costa and Pelissari, 2016; Klein et al., 2019). Therefore, all activities and materials in the learning environment will affect learning (Demiralp, 2007).

The realization of more adequate and high level learning in teaching can be achieved by providing information in different ways (such as audio-visual) in the teaching environment and creating multimedia in teaching (Gunawardhana and Palaniappan, 2016). The teaching environment should be supported with materials in order to achieve this and to make the teaching more concrete and simplify the subject. Materials used in teaching are one of the basic elements of quality education (Naude and Meier, 2019). Materials selected in accordance with the subject and purpose bring the taught subject alive, enrich the teaching process and increase learning. Materials also broaden students' interests and increase their motivation (Demiralp, 2007; Fryer et al., 2017).

The use of the materials can increase the students' interest in the course and enable them to make inquiries about the subject by ensuring their participation in the lesson. As the presentation of audio-visual tools in the form of materials suitable for the lesson will appeal more to the sensory organs, so will the learning be permanent. Thus, learning will take place according to the constructivist approach rather than rote learning and forgetting will be too late (Opare et al., 2018; Afrizon et al., 2019).

In biology teaching, biology concepts, many of which are abstract and difficult to learn, should be learned in an effective, meaningful and permanent way, and new methods should be used by moving away from classical methods in order to prevent mislearning (Cakmak and Havedanli, 2004). Students' learning about the concepts and their ability to establish a relationship between the concepts depend on the preferred teaching method and the use of appropriate material selected for that method (Ozalp, 2006).

According to the constructivist approach, since students need to be active in the process of structuring information, it is important to use visual tools that can add students to the lesson, create discussion environments, and enable them to learn more meaningfully (Balim et al., 2008; Marques et al., 2018). It is thought that students perceive abstract and incomprehensible concepts such as biology concepts better with the help of visual tools (diagram, graphic, photograph, picture). In addition, it is known that visual memory is more easily remembered than verbal memory, and all kinds of visual communication tools such as drawing, diagrams and pictures help students to establish a neural information network and improve visual memory (Ozalp, 2006; Tyler-Wood et al., 2018).

According to recent researches, it is stated that cartoons can be used as visual education material in student-centered education with a constructivist learning approach (Hourani et al., 2017; Liang et al., 2019).

Cartoons are art products in which people and things are drawn in a funny way by exaggerating, humor is made on the line, and that can appeal to all age groups, including entertainment, humor, satire, thinking and visuality. It is widely used in scientific and technical issues as it enables information and messages to be delivered easily and permanently (Ozsahin, 2009; Balim et al., 2016; Al-Rabaani and Al-AAmri, 2017). Therefore, cartoons should be used scientifically in the field of education in order to ensure the permanence and easy transfer of the information required for learning.

In addition, cartoons can be designed as materials that can be used to teach the concepts to students in a correct and fun way (Kete et al., 2009). Concept cartoons are one of the visual tools that we can prepare and use on the basis of the constructivist approach in order to create permanent learning by ensuring the active participation of students in the course during the learning and teaching process in biology education. It is provided by speaking in balloons and presenting it with very little use of language (Evrekli and Balim, 2010; Yin et al., 2016; Akbas and Kilic, 2019).

In concept cartoons, the events encountered in daily life are the presentation of two or more characters' conversations on problems or ideas in the form of speech bubbles. (Inel et al., 2009). While cartoons are used to make individuals laugh, concept cartoons are used to entertain students and to question their knowledge, to direct them to think and discuss (Evrekli and Balim, 2010; Sengul and Uner, 2010).

Concept cartoons, which can be used as tools to develop the conceptual event, are seen to create an efficient teaching environment in a social process. (Kete et al., 2009; Birisci et al., 2010).

In a study realized by Inel et al., (2009), that the students' opinions were positive about the use of concept cartoons and the concept cartoons increase the understanding and the interests of the lessons, researchers found that cartoons positively affect the academic achievement of students and made a significant difference.

In the light of the utilized literature, this study is the one that tries to show that concept cartoons, which are generally entertaining and also effective visual materials, can be used in a constructivist approach in education, especially in biology teaching, teachers can benefit from the learning-teaching environment in order to create effective, efficient and permanent learning.

The purpose of this study is to determine the effect of teaching with concept cartoons in biology teaching on student achievement and the permanence of learned knowledge based on the constructivist learning approach. Problems and sub-questions were determined within the framework of this aim.

The problem of the study is, is there a significant difference between the learning levels of the experimental group taught with concept cartoons and the traditional control group in the "Ecosystem Ecology" unit of the secondary school biology course (10th grade)?

1. What is the effect of teaching with concept cartoons on student achievement?
2. What is the effect of concept cartoons on the permanence of knowledge?

2. Materials and methods

In the study, the control group pre-test, post-test quasi-experimental research method was used. The quasi-experimental method is the most commonly used method in educational research when it is not possible to keep all variables under control (Gul and Yesilyurt, 2011).

2.1. Study group

The 10th grade students studying at Kazım Karabekir Technical and Industrial Vocational High School in Yunus Emre district of Erzurum province were selected as the study group. The universe of the study covers the 10th grade digital classes with biology lessons. The study was applied on 49 students in two of the 10th digital classes. Two classes were determined with the permission of the school principal and biology teachers. In the study, achievement test was applied to two classes in the school selected with the appropriate sampling method before starting the actual application and it was determined that there was no significant difference between the groups. Accordingly, a class was selected by random method as a sample from these two classes, which were found to be equivalent, and the experimental group was formed by showing the cartoons in the form of slides in a computerized environment and applying lectures with cartoons. In the other class, the control group was formed by applying the method of direct instruction without showing the cartoons.

2.2. Data collection tool

In the study, biology achievement tests including three-stage pre-test, post-test and permanence test consisting of 25 questions on the subject of "Ecosystem Ecology (especially Ecosystem Structure)", which is the third unit of the 10th grade biology course, from the preparation course books of the student Selection Examination (ÖSS), were prepared and used. The correct answer for each item was determined as 4 points and the maximum score to be obtained from the test was 100. The tests prepared were examined by course teachers, science educators and measurement experts.

2.3. Research application

This study includes a seven-week application. The study was applied to a total of 49 numerical class students, including 30 experimental groups and 19 control groups. In both groups, the lesson was taught by the same biology teacher and practices related to the lesson were made.

Pre-test, post-test and permanence test were applied to both experimental and control groups. A pre-test was applied to both groups before the lesson was taught. Then, the control group was taught with the traditional teaching method, which is direct instruction.

In the experimental group, the researched concept cartoons were given to the group as a material with the method of teaching, and the lesson was presented as slides supported with cartoons, and was actively processed in the form of questions and answers based on the constructivist approach. It was administered to both groups during normal class hours. In the experimental group, in which the method of lecturing with cartoons was applied, it was observed that the lesson was fun and enjoyable, every student attended to the lesson, students read and understand cartoons and laughed during the lesson, and the lesson was handled in a pleasant way.

After the topic of the lesson was finished, a post-test was applied to both groups. After four weeks, a statistical analysis and evaluation of the data was performed by applying a retention test to both groups.

2.4. Analysis of the data

The analysis of the data was done through the SPSS 15 package program. One-way analysis of variance was used to determine the effects of independent variables on the dependent variable (Biology Field Knowledge Test Result). A significance level of 0.05 was adopted in the comments regarding whether the differences between groups are significant or not.

3. Results

Random pre-test and post-test achievement tests were applied to the control group and the experimental group in the analysis of the findings on the achievements of the students in the biology lesson Ecosystem Ecology with the concept cartoons. In addition, the retention test was applied to the groups after 4 weeks. The data were analyzed by comparison method.

In the study, pre-test averages, standard deviations of the experimental and control groups for the success test are given in Table 1, post-test averages and standard deviations are given in Table 2. The retention test averages and standard deviations applied to the groups four weeks after the pre-test and post-test applications are given in Table 3. In addition, the pre-test, post-test and retention test analysis results regarding the achievement test of the students in the experimental and control groups are compared in Table 4.

Table 1
Descriptive statistics table of the experimental and control groups for the pretest.

Groups	**N**	$\overline{X}$	**SS**
Experiment	30	23.26	9.91
Control	19	20.21	9.84
t	**S. D**		**Importance Level (p)**
1.054	47		0.297

An independent sample t test was conducted to determine whether there was a significant difference between the experimental and control groups in terms of pre-test scores. According to the analysis results, it was revealed that there was no significant difference (p> 0.05) between the groups.

Table 2
Descriptive statistics table of the experimental and control groups belonging to the posttest.

Groups	**N**	$\overline{X}$	**SS**
Experiment	30	60.66	11.02
Control	19	34.31	12.45
t	**S. D**		**Importance Level (p)**
7.752	47		0.000

An independent sample t test was conducted to determine whether there was a significant difference between the experimental and control groups in terms of posttest scores. According to the analysis results, it was revealed that there was a significant difference (p <0.05) between the groups.

Table 3
Descriptive statistics table of the retention test of the experimental and control groups.

Groups	**N**	$\overline{X}$	**SS**
Experiment	30	47.06	13.06
Control	19	32.63	9.91
t	**S. D**		**Importance Level (p)**
4.118	47		0.000

In order to determine whether there was a significant difference between the experimental and control groups in terms of retention test scores, an independent sample t test was conducted. According to the analysis results, it was revealed that there was a significant difference (p <0.05) between the groups.

Table 4
Comparison table of pretest, posttest and permanence test analysis results for the achievement test of students in the experimental and control groups.

Groups	**Pretest Average**	**Posttest Average**	**Permanence-test Average**
Experiment	23.26 (DGÖTO)	60.66 (EGPOTA)	47.06 (DGKTO)
Control	20.21 (KGÖTO)	34.31 (KGSTO)	32.63 (KGKTO)

Before the application, it was investigated whether there is a significant difference between the groups in terms of student achievement. As seen in Table 4, the results obtained from the analyzes performed before the actual application revealed that there was no statistically significant difference between the experimental and control groups in terms of biology knowledge success at the beginning. Table 4 shows that the success of the experimental group increased after the teaching method with concept cartoons was applied and the information was more permanent.

Additionally, the pre-test average (EGPrTA) of the experimental group was calculated as 23.2667, while the pre-test average (CGPrTA) of the control group was calculated as 20.2105. These results show that the knowledge levels of the groups before the application were very close to each other and that there was no significant difference between the groups.

After the application, the posttest average (EGPoTA) of the experimental group was 60.6667, while the posttest average (CGPoTA) of the control group was 34.3158. As a result of the analysis, an average significant difference was found between the experimental and control groups (CGPoTA<EGPoTA). This result shows that the teaching practices carried out with concept cartoons are more effective in teaching the "Ecosystem Ecology" unit of the biology lesson than traditional teaching.

In addition, according to the results of the permanence test applied and shown in Table 4, despite the four weeks after the application, the control group permanence test average (CGPTA) was calculated as 32.6316, while the experimental group permanence test average (EGPTA) was calculated as 47.0667 (CGPTA<EGPTA). As a result of this retention test analysis, it was determined that concept cartoons are a suitable material for teaching and provide permanent traced learning. This result shows that teaching with concept cartoons has a positive effect on students' learning biology knowledge compared to the traditional method.

3. Discussion

Raising individuals who produce knowledge and use the information they produce will be possible primarily by learning to learn. Learning to learn requires understanding of learning strategies (Tasdemir and Tay, 2007).

Learning takes place through the mental participation of the individual himself. Learning does not occur by simply storing information in the student's brain through the narrative method (Anderson and Mack, 2019; Ball, 2020). For this reason, a contemporary teacher knows the importance of using new methods and techniques in order to gain new behaviors to his students and tries to show his students to practice by learning new learning strategies. Compared to the traditional teacher, the modern teacher provides a learning environment that appeals to more sensory organs, meeting the different learning needs of the students and providing a multiple learning environment (Calalb, 2017; Yagci and Guneyli, 2018). It brings students closer to the lesson, arouses their interest, simplifies the subject and makes it easier to understand. A teacher using only one method and technique cannot be successful. As much as possible, teachers should use materials and tools that attract attention, ensure active participation of the student in the lesson, and appeal to the student's many senses, such as slide pictures, concept maps and concept cartoons. Permanent learning will be achieved in the learning and teaching process with these visual materials (Wijaya and Bunau, 2017; Erdem and Koc, 2019; Sáez-López et al., 2019; Woldeamanuel et al., 2020).

The problem of the study is determined as "Is there a significant difference between the learning levels of the experimental group that is taught with concept cartoons in the Ecosystem Ecology unit and the control group that has traditional education?". In line with the solution of the problem in question, "What is the effect of teaching with concept cartoons on student achievement? What is the effect of teaching with concept cartoons on the retention of the learned information?" Answers to these sub-questions were sought.

As a result of the analysis; It was observed that there was no significant difference between the pretest achievement test scores of the experimental and control groups and the groups were considered to be relatively equal. After the application, the achievement test was applied to the experimental and control groups as a post test and it was determined that there was a significant difference between the posttest success scores of the groups. In line with this finding, it can be said that teaching with concept cartoons in the learning and teaching environment causes a significant difference in students "achievement and that concept cartoons affect students" success positively.

In order to examine the effect of teaching with concept cartoons on the retention of the learned information, a retention test was applied to the experimental and control groups 4 weeks after the application. As a result of the retention achievement test analysis, it can be said that there is a significant difference between the experimental group and the control group, and teaching with concept cartoons provides the permanence of the learned information and positively contributes to permanent learning.

In the study by Kete et al. (2009), "Attitudes of pre-service teachers about the use of cartoons in their worksheets", it has been revealed that the analysis findings of the attitudes of teacher candidates that cartoons have an effect on learning, create permanent knowledge, create an environment for curiosity, thinking, research and discussion, provide motivation, and are entertaining and instructive. It is seen that the teacher candidates showed a high attitude to the item "I quickly perceive the message given by cartoons" by 87%, to the item cartoons are a good educational tool, 73%, to the item cartoons reinforce the meaning of the topic, 84% to the item that cartoons are entertaining and instructive, and 100% to the item I never forget the subject about cartoons. In the study of Inel et al. (2009) on the use of concept cartoons in science teaching, students stated that they wanted concept cartoons to be used in science lessons, that concept cartoons are beneficial in many ways, help them understand the lesson and increase their interest in the lesson, such as how the lesson is handled differently and the concept cartoons are illustrated. They stated that they liked concept cartoons due to their characteristics. The views of the students in the findings of this study and the teachers' views in the study of Kete et al. (2009) support each other and are in line with the findings of our study.

Ozsahin's (2009) "Teaching Geography with Cartoons" supported the students' opinions that the cartoons would be used in the lesson in the survey conducted on 12th grade students. More than 75% of the students advocated the use of cartoons and other visual materials in lessons, and more than 80% of the students argued that if their teachers show cartoons related to the subject in lessons, it will make the lessons more enjoyable and they can learn the subjects more comfortably. These findings are also in parallel with the studies conducted.

Balim et al., (2008), in the study "The effect of the use of concept cartoons in science teaching on students' academic achievement and inquisitive learning skills perceptions" is that the independent variable is concept cartoons, there is no significant difference between the experimental group and the control group posttest academic achievement scores, the analysis. As a result, it was determined that concept cartoons alone do not have any effect on academic achievement. Initially, it was observed that this finding did not support our study. However, it was stated in the study that when concept cartoons are used together with different methods and techniques, they can contribute positively to academic success. In line with this view, in the practice of our study, it was determined that the concept cartoons had a positive effect on teaching with the active participation of the student, in the form of questions and answers based on the constructivist approach supported by slide display on the computer and supported by findings of Balim et al., (2008). The opinion was reached by analyzing the findings obtained.

In the research findings of the study named "The effect of the use of cartoons on students 'academic achievement and their views on the lesson" in which the effectiveness of the use of cartoons in Social Studies lesson was investigated, it was determined that the use of cartoons had a positive effect on students' academic achievement (Akengin and Ibrahimoglu, 2010).

In study of Evrekli and Balim (2010), it was investigated whether there was a significant difference between the post-test academic achievement scores of the students in the experimental group studying with activities based on the use of concept cartoons and the students in the control group studying only with the "Science and Technology" curriculum, and as a result of the analysis findings, experimental and control, it was determined that there is a significant difference between the posttest academic achievement scores of the groups. In line with this finding, it was stated that the activities based on the use of concept cartoons caused a significant difference in the academic achievement of students only according to the activities in the science and technology curriculum.

Erdogan and Ozsevgec (2012) developed student-centered materials for "learning the greenhouse effect and global warming" and after the application these materials, they found significant differences between the pre-test and post-test mean scores of the students. They concluded that the concept cartoons used ensured active participation by placing the student at the center and effective on eliminating students' misconceptions.

Cinici et al., (2014) were found a significant difference in favor of the experimental group between the achievement levels of the students in Science and Technology lesson in the eighth class "Cell Division and Inheritance" unit. They stated that the students liked participating in collaborative studies and discussions in the argument-generating process, and they found the use of cartoons was funny. Additionally, they observed that students had the opportunity to directly participate in the thinking, discussion and questioning-style activities and they had the opportunity to learning by applying cognitive and social activities related to the scientific process.

Kaplan et al., (2014) observed that the students had misconceptions about square root numbers in mathematic class. They concluded that the concept cartoons and traditional teaching were effective in eliminating misconceptions. They also stated that while traditional teaching only caused a positive change in comparing square-rooted numbers, concept cartoons had a positive effect on all achievements. In addition, their results showed that concept cartoons were more effective in comparing concept cartoons and traditional teaching.

In another study, it was proven that peer learning with concept cartoons enhance students' interest in positive manner. According to researchers' obtained data, 39.84% of the respondent people were agreed to this. Also, analysis were indicated that PLCC can enhance the interests of the students (Yin et al., 2016). In a recent study realized by Al-Rabaani and Al-AAmri, (2017) showed that in lessons of social studies, using cartoons has significantly increased students' water awareness level, when compared to the other traditional methods. They added that the cartoons create a learning environment to the students and more interest for looking and reading the materials as a result of the included satire symbols irony, comics, in the used cartoons. Lately, Dayan, 2021 studied the metaphors produced by primary school 3rd grade students for concept cartoons, which are a teaching material in the "Life Studies" course. Students produced 77 metaphors for concept cartoons, and these metaphors were discussed under 10 categories. These categories are; description, fun, information source, life, object, educator/instructor, place, person and profession, action and others.

The researcher concluded that concept cartoons, one of the metaphors produced by the students, are justifications emphasizing that they are entertaining and informative.

As a result of the research conducted in order to create a stimulating effect for the use of cartoons in biology teaching by describing different perspectives on the use of concept cartoons as visual material in education and training in line with the researches and perspectives in the literature; The use of concept cartoons in biology teaching in a computer-aided environment in the form of slideshows contributes positively to student success, increases students' interest in the lesson, makes the lesson environment entertaining and instructive, enables the student to participate actively in the lesson, encourages students to learn more quickly and more permanently. And it can be said that it effects students positively towards biology subjects.

Conflict of interest: The authors declare that they have no conflict of interests.

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Review article

Modern agriculture and challenges

Abdulgani Devlet*[1]

[1] *Bilecik Seyh Edebali University, Faculty of Agriculture and Natural Science, Department of Field Crops, 11230, Bilecik, Turkey*

Abstract

In this review, it was tried to find out the challenges faced by modern agriculture and the relationship between related industries and enterprises. With the infection control of the Covid-19 pandemic, the prevention and treatment of natural disasters and the healthy development of human society have become common concerns. The focus is on human health and the importance of the agricultural and industrial sectors, including food health, food security, and economic development. As the demand is also updating, in the development process of these three basic problems, we should not only review the previous planning, overcome and prevent the existing problems but also do more research and exploration with the new planning. These three topics are important issues that need to be dealt with urgently in a person's or a country's or international relations. Scientists strive to help farmers understand how modern agriculture is and must be a part of the solution for these challenges.

Keywords: *Challenges; development; industry; modern agriculture; trade*

1. Introduction

According to the final report of the Food and Agriculture Organization of the United Nations (FAO) in 2020, although the Covid-19 pandemic has different degrees of impact in all food sectors, the agricultural and food sectors have advantages in this respect, so other sectors are not as good as others in resisting the epidemic. The report predicts that the production and market trends of grain, oil crops, meat, dairy products, fish, and sugar will become the world's largest trade grain commodity in 2020-2021. The situation of cereal supply and demand is good. FAO's early forecast shows that global grain production in 2020 will exceed 2.6% of the previous year. It is estimated that world cereal trade volume will reach 433 million tons in 2020/21, an increase of 2.2% (9.4 million tons) compared with that in 2019/20, and will reach a new historical high due to the expected expansion of all major cereal trade (FAO, 2020a).

In order to meet the increasing demand up to 2050, it is estimated that global agricultural production will need to increase by 70% (Bruinsma, 2009). Improving rural poverty and community farms in urban suburbs is one of the measures to achieve food security in developing countries. In order to promote global food security, low-income countries need to increase their quantity and quality of food production in order to reduce their vulnerability. In many food-dependent low-income countries around the world, especially in Africa, there are huge differences in production (FAO, 2011a).

Sustainable agricultural mechanization is a key strategy to achieve long-term growth of agricultural production in all aspects, including reducing the coolie of small-scale farmers, improving the timeliness of agricultural operations and improving the efficiency of input and use. In the long run, mechanization will contribute to the sustainable strengthening of production systems and the establishment of an agricultural sector more resilient to increasingly extreme and unpredictable climate events (FAO, 2017). Small farms are at the center of the strategy of food supply chain. Small-scale agriculture will continue to improve productivity and increase the local food supply. In this way, we can not only effectively reduce poverty, but also make a significant contribution to economic

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* Corresponding author.
E-mail address: abdulgani.devlet@bilecik.edu.tr (A. Devlet).
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development. Around 75% of the world's people, most of them living in rural areas are still extremely poor, live with hunger and fear starvation. Small-scale producers and landless households, a large part of people's income directly depends on agriculture or is engaged in agriculture based activities and the rest are small business owners related to agriculture, such as processing, machinery, storage, seeds, feed or fertilizer. A large number of starving people in poor countries only depend on agriculture for a living, but for a long time, their input is seriously insufficient, which hinders the overall productivity of agriculture. Lack of investment also reduces farmers' ability to cope with price fluctuations and external shocks, including those related to weather and the economy. Climate change is affecting farmers' livelihoods and food security around the world. There are clear signs that there may be underinvestment, which will have a huge impact on poverty reduction. For example, in the World Bank 2008, the quantitative analysis of the effect of poverty reduction shows that the growth of agricultural GDP (gross domestic product) is faster than that of external GDP (World Bank, 2008). Sustainable production of healthy livestock with nutrition and food for everyone is crucial. However, there is no specialized organization that can deal with the risk of specific diseases on time, and prevent, control and eliminate livestock diseases. Therefore, it is not only necessary to be on time in view of the seriousness of disease problems and problems in medicine, public health, veterinary medicine, entomology and environment, but also need to carry out firm international coordination and cooperation to develop and eliminate livestock diseases within the framework of the concept of "one health" Implementing the global strategy for collective health protection (FAO, 2011b). It mainly includes locusts, armyworms and fruit flies, which pose a major threat to agricultural and animal husbandry resources and livelihoods, as well as wheat, coffee and soybean rust, banana fusarium wilt, cassava and corn virus diseases, which spread rapidly, threatening neighboring countries, regions and continents. This disease seriously affects nearly 70 countries in the world, which is highly infectious. More than 80% of sheep and goats and more than 330 million people live in the world's poorest areas, causing losses of US $1.5 billion to US $2 billion per year, many of whom rely on small ruminants for their livelihood (FAO and OIE, 2015).

As a labor-intensive sector, agriculture can certainly absorb underutilized labor. For example, some farmers and rural workers have no land or too little income. In addition, agricultural growth has led to a decline in food prices, a doubling of the role of the local economy, and an increase in rural wages (Schmidhuber and Bruinsma, 2011). Therefore, the necessary condition of food security is not only to make full use of the existing natural resources, but also to invest in small farmers' agriculture and promote the development of a fair economy.

FAO published a paper entitled "how to feed the world by 2050" at an expert meeting in Rome in 2009, including the main conclusions of the meeting (FAO, 2016). A large part of the world's agricultural production is grain production. By 2050, according to the data of FAO, the world's total grain output is expected to reach 3 billion tons (Alexandratos and Bruinsma, 2012). It is estimated that global meat production will reach 429 million tons in 2001 and 470 million tons in 2050, which is more than twice the increase (Scollan et al., 2010).

The commercial and nutritional quality of fruits and vegetables depends on a range of characteristics, attributes and characteristics (Schroder, 2003). The identification of commercial quality standards includes origin, freshness, cleanliness, appearance, hardness, consistency, no damage, color, no disease, aroma, texture, size and shape (UNECE, 2007). Nutritional quality, essential nutrients such as carbohydrates, amino acids and fatty acids are related to bioactive compounds such as phytosterols, dietary fiber, vitamins, carotenoids, phenolic acids, glucosinolates and flavonoids. In the process of delivery, quality will be affected by various activities in the supply chain. For example, the sales quality of fresh vegetables decreased due to mechanical damage caused by handling and transportation vibration during transportation. The next doubling of food production will be achieved through land reduction per capita and water shortage. In order to meet the needs of the future population, the increase of food production is faced with some challenges, such as the increase of high protein food sources (Singh-Ackbarali and Maharaj, 2017). In food applications, it is most important to be able to quickly and correctly detect harmful and unhealthy (off-label) animal and plant substances in processed food (Yancy et al., 2008). The main goal of all food safety organizations is to ensure the food safety of consumers in an all-round way (Cutarelli et al., 2014).

All in all, in human history, the long-term expansion of population has been limited by food supply and disease constraints (Diamond, 1997). The domestication of animals and plants has further promoted the development of human society, caused a large number of people, and increased the risk of disasters and major disease outbreaks (pandemic). The recent global pandemic of Covid-19 is caused by the emergence of a disease caused by severe acute respiratory syndrome coronavirus 2 (Sars-Cov-2). Moreover, it has caused great interference and losses to economic activities and severely restricted international cooperation, exchanges and exchanges. The pandemic exacerbates new challenges to food security posed by climate change and major conflicts, both of which are major factors contributing to overall food insecurity. Plant scientists need to identify investment in innovation and change measures to deal with the pandemic. Ensuring supply through the use of modernization and automation at all stages of the food production system will exacerbate concerns and challenges about labor shortages and food safety. Responding to transport and trade disruptions to support food production close to the point of consumption may facilitate accelerated efforts to develop protective crops. Both trends will increase the demand for new crop varieties to meet the growing demand of consumers, better promote more research work, including successful prevention and treatment of epidemics, and accelerate the application of emerging plant breeding technologies in these rapidly developing modern agricultural environments (Henry, 2019; Genc, 2020; Uras, 2021).

Global value chain (GVS) refers to the sharing of international production, a phenomenon that divides production into activities and tasks in different countries. Global value chain accounts for half of the total trade volume, which promotes the surge of international trade and creates unprecedented economic integration: some developing countries are becoming net importing countries and growing constantly: Indonesia, Nigeria, Algeria, Pakistan, Iran and the Republic of Korea are such cases, which make up for the lack of net import growth of developed countries. All over the world, governments are actively and openly intervening in the economy to promote innovation, create new technologies and cultivate cutting-edge industries. These interventions can have a positive or negative impact, especially in today's highly interconnected global economy. On the one hand, they can expand knowledge, increase productivity and disseminate basic tools for global growth and development. But on the other hand, they may also distort trade, transfer investment, benefit one economy and harm the interests of other economies. More than ever, international cooperation and rules are needed to ensure that governments' new focus on innovation and technology policies can maximize positive spillover effects, minimize negative spillover effects, and ensure that competition for technological leadership does not evolve into a struggle for technological dominance (WTO, 2020a). The global financial crisis in 2008 also brought a serious threat, adding pressure to the trade led growth model. New technology can shorten the distance between production and consumers and reduce the demand for labor. With the development of industrial and agricultural trade and technological change, global value chain can continue to promote growth. The premise is that developing countries implement more in-depth reform and innovation, and promote participation in global value chains. Industrial countries pursue open and predictable policies, and they need to strengthen and resume multilateral cooperation. With the reduction of trade and communication costs, the development of new products and the improvement of productivity, developing countries should accelerate the reform of trade and investment and improve the level of connectivity. It is conducive to promoting the development of global value chain and making it a sustainable and inclusive development force. (World Bank, 2020).

2. Agriculture and challenges

With the rapid development of cities, the urbanization of the world has entered unprecedented progress, 55% of the world's population is urban, and the border population has been growing by about 7.5 billion since the early 1960s (FAO et al., 2018). In Asia and Africa, urban population growth has reached 90% (UN DESA, 2018). By 2050, 2.5 billion people are expected to live in urban areas. Unprecedented urban development is now taking place all over the world, and the urban population accounts for more than half of the global population. Access to adequate, safe, nutritious and cash regulated properties in urban areas poses specific food security and nutrition challenges. The actual distance between grain producing areas and consumers, the lack of transport options, the fluctuation of grain prices, the concentration of power in global grain trade, the impact of climate, and the failure of the safety net of low-income urban residents, especially in times of crisis, often limit the access to food (FAO, 2019). In order to meet the growing world population, the production of adequate food has become the primary and sustained challenge for agriculture all over the world. The share of agriculture in total production and employment is declining at different rates, and the challenges are different in different regions. The second challenge facing global agriculture is to develop new technologies, policies and institutions that will help to realize the full potential of agriculture as an engine of growth. Although agricultural investment and technological innovation are increasing productivity, it is disconcertingly low that output growth has slowed down. In order to reduce the loss and waste of grain in agricultural output, the goal of increasing production can be achieved. However, the degradation of natural resources, the loss of biodiversity and the spread of plant and animal diseases and pests across the border have hindered the necessary acceleration of productivity growth, and some of them have become resistant to antimicrobial agents. Developing a new set of technologies, incentives and policies to encourage small-scale farmers to attach importance to long-term management of natural resources, and improving the productivity and profitability of small-scale farmers are closely related to improving the management of natural resources in developing countries (FAO, 2017). Based on indigenous and traditional knowledge, the establishment of agroecology, agroforestry, climate intelligent agriculture and protective agriculture is a process of "holistic" transformation. To solve the problem of climate change and the aggravation of natural disasters affects all ecosystems and all aspects of human life, so it needs to be realized through the progress of new technologies, coupled with the sharp reduction of economic scope and the use of agricultural fossil fuels. It is also necessary to strengthen international exchanges and cooperation to comprehensively prevent emerging cross-border agricultural and food system threats, such as pests and diseases. Strengthen the innovation system based on the conservation of natural resources to improve productivity. However, there is a need to respond to growing demand, climate and soil changes, mainly the risk and pressure of human interference with agricultural production systems (FAO, 2019).

Floods affect human survival, property and economic activities in many ways. There has been a lot of research on how to prevent, mitigate, manage and deal with the clean-up and recovery phases. Although the flood frequency is not high and the occurrence time is short, the damage to property and houses can cause a serious economic burden. By promoting and supporting integrated watershed and plain management while focusing on the establishment of good drainage systems and forest protection, the capacity and occurrence of catastrophic floods can also be fully mitigated and prevented (Murnane, 2004). Climate change and drought affect all regions of the world. It is not only a climatic feature, but also a temporary condition caused by water shortage, which can occur under almost any climatic condition. The definition of drought occurrence occurs when the rainfall is lower than the long-term average rainfall of a certain place in theory, and the location is very important (FAO, 2017; 2019).

From 2010 to 2014, different parts of the world were hit by drought, and agriculture, industry and commerce posed great challenges. Climate change may lead to more frequent and severe droughts. In dry land, semi-arid areas, which usually have more population and more economic and social activities, are more affected by drought. Based on the analysis of historical drought trends, there are significant regional differences in drought and its impact (Field and Barros, 2014). The International Food and Agriculture Organization (FAO) said that compared with the global food price crisis from 2007 to 2008, the global food production prospect is optimistic and the food situation is better, which has become a new problem of food access. The price is low, the inventory is high, there are many import and export countries, and the trade base is broad. Due to the Covid-19 incident, the economic growth rate dropped sharply, which restricted people's ability to obtain adequate and nutritious food. Policymakers should be prepared for similar global crises and gain more experience and insight ahead of time. At present, the Covid-19 pandemic has also led to the loss of researchers, the closure of many research laboratories, the cancellation of many global research conferences and the reduction of direct contact between researchers, which has partially destroyed the existing agricultural and food research system (Capell et al., 2020; Tokel, 2021).

In different regions and countries, there are great differences in planting systems and agricultural technology use. Under the premise of international exchanges, new agricultural technologies and reforms have been widely spread all over the world. The socio-economic, agronomic and technical challenges faced by agricultural measures are due to the limited cost and lack of skills, which are known as socio-economic barriers. There are many technical barriers, including machinery, sensors, GPS, software and remote sensing. However, the adoption of precision agriculture and other measures will gradually eliminate the obstacles encountered, which will play an important role in the future agricultural system (Robert, 2002). However, in recent years, the introduction of high-tech technologies, such as automatic fertilizing devices, autonomous agricultural machinery and a series of computer software for managing various production systems, has generally taken effect everywhere (Gebbers and Adamchuk, 2010). Of course, precision agriculture is a promising form of agriculture (Mulla and Khosla, 2015). These advances in agricultural modernization have also brought many benefits to farmers, including increased productivity and the resulting profitability, farm quality, clean environment, food safety and sustainability, and households and consumers who may be affected globally. At present, the main challenges are to take measures to identify new approaches to crop diseases and pests, reduce pesticides and agricultural practices and activities harmful to humans and the environment, and redesign agricultural management models in the information age (Nyaga et al., 2021).

At that time, more than 840 million people are suffering from malnutrition, of which Africa and South Asia account for a high proportion. Around the world, most of the 1.3 billion people who are engaged in agriculture live on less than $1 a day. The worry is that the rate of degradation of natural resources is accelerating (McCalla, 2001). Although it is too early to assess the full impact of the blockade and other containment measures, the report estimates that at least 83 million people, and possibly as many as 132 million people, will starve in 2020 due to the recession triggered by the Covid-19 (WHO, 2020). The map of hunger 2020 depicts the prevalence of malnutrition in all countries in 2017-2019 - if current trends continue, by 2030, the number of starving people will reach 840 million (WFP, 2020). “Green Bay Crops” are mainly common garden vegetables, as well as potatoes, some oats and spring wheat. People on the grassland grow many small grains, such as wheat, barley, oats, potatoes and onions. Grain was one of the most important commodities in the trade between the eastern Mediterranean and western city-states in the first half of the 14th century and the 15th century. Food is vital to survival and an important part of the trade. International trade was the focus of the early construction of the Ottoman Empire. The trade relationship between European merchants and Muslim merchants is discussed from the development of the Ottoman Empire in 1300 to Constantinople in 1453. The economic development of the early Ottoman countries and the expansion of Ottoman territory provide a rare insight into their economic aspirations and eventual integration into the Mediterranean basin economy, and clarify the close relationship between Muslims, agriculture and trade (Fleet, 1999). White settlers in fur trading villages grow gardens in the hope of feeding their farmers and trading any additional agricultural products (Apps, 2015).

In 1994, the first genetically modified food approved for marketing was yellow tomato, which successfully inserted an antisense gene delaying ripening and had a longer shelf life. By 2000, transgenic crops such as potato, Bt corn, Bt cotton, glyphosate-tolerant soybean and golden rice have been completed (James, 2011; Tokel et al., 2021). Advances in biotechnology have created contradictions on a wider range of social, ethical, religious and economic issues. Many environmental organizations and consumers are strongly concerned about the direct and long-term effects of genetically modified organisms on human health. At the same time, environmental risks, such as the reduction of biodiversity, the spread of superbacteria, gene leakage and agricultural sustainability of genetically modified crops, were emphasized (RAFI, 2000). The area of genetically modified crops in the world increased from 1.7 million hectares in 1996 to 52.6 million hectares in 2001. In 2001, the United States (35.7 million hectares), Argentina (11.8 million hectares), Canada (3.2 million hectares) and China (1.5 million hectares) planted 99%. Transgenic companies began to invest a lot of money in the research and development of transgenic crops and determined to expand the transgenic market. Biotech crops have been commercialized for 22 years. In 2018, 17 million farmers in 26 countries planted 191.7 million hectares of biotech crops. Compared with 1996, the planting area of 191.7 million hectares in 2018 increased by about 113 times. Therefore, the fastest crop technology in modern agricultural history is considered to be Biotechnology Crops (ISAAA, 2018).

Farm classification is the process of classifying each census farm according to the main production types. This is achieved by investigating the potential revenue from crop and livestock inventories to estimate and identify the products or product groups that account for the majority of the estimated revenue. Total agricultural revenue includes all agricultural sales, programs and refunds, sales committee payments, tax rebates for goods and services, customs revenue, cooperative dividends, and agricultural product revenue. The renewal and innovation of agriculture promote the development of new markets and the opportunities brought by new technologies (Planscape, 2003). The innovative approach to food supply is an iterative cycle, including the design and construction of target genotypes (using plant biotechnology equipped with advanced genomics and gene editing) and the production environment (the most cost-effective engineering environment). Sustainable and reliable modern agricultural production can be achieved regardless of the recurrence of challenges such as climate change or disasters such as pandemic (Pouvreau et al., 2018).

Although economic difficulties lead to low population growth rates in developed countries, the adverse impact of Covid-19 on the global economy may also lead to new risks of accelerated population growth, thus greatly increasing food insecurity (Genc, 2020). Poverty is also a major factor leading to rapid population growth in developing countries (Van Bavel, 2013).

The environmental performance index 2020 ranks 24 performance indicators of 10 problem categories in 180 countries. The sustainable development of agricultural industrialization in ecological countries provides new employment opportunities for researchers, scientists, biotechnology experts, veterinarians, farmworkers and development technicians. At present, the introduction of genetically modified organisms (GMOs) has attracted some attention, and the planting area of these organisms is expanding every year (Arvas and Kaya, 2019). The latest technological innovations in agronomy, transgenic plants, chemical pesticides and chemical fertilizers have accelerated synchronous development and improved the quality and yield. Due to the increase of human population and food demand, genetically engineered plants and their products are obtained by using recombinant DNA technology, which is the result of technological development in the last 25 years (Arvas and Kocacaliskan, 2020).

3. Agriculture and industry

In recent decades, agriculture is characterized by a large number of purchasing inputs, which inevitably depends on the industrial sector. Industrial benefits of agricultural related enterprises have increased significantly, especially due to investment opportunities in post-harvest processing of crops, such as food processing and exports (Satterthwaite et al., 2010). The impact of agriculture on production in the industrial sector has increased over time. Before the 1980s, the real GDP of the industrial sector was more flexible than that of the agricultural sector, and the relative importance of agriculture and industry was higher. Agriculture is an integral part of the process of industrial development, and the mutual promotion and development of industry and agriculture is a complete process. Agriculture contributes a lot to the whole industry, especially to the economy. For example, the role of industry in providing inputs to modern agriculture, especially after the green revolution, and in expanding the demand for wage products, need hardly be mentioned (Satyasai et al., 1999).

The meat industry accounts for a high proportion of the world's food industry in many countries, which plays an important role in the global economy. The poultry (13.6 kg/year) were the largest per capita meat consumption in the world (Silva et al., 2016; Zocca et al., 2018). At the same time of consumer demand, refrigeration system is the key to obtain product stability and sensory characteristics, and prevent the development and change of meat ingredients, bacteria and microorganisms in the meat industry (Savell et al., 2005). Slaughterhouses (European Commission, 2003) and meat processing industries (Ramirez et al., 2006; Alcázar-Ortega et al., 2012) consume 60% - 90% and 40% - 50% of cooling system power, respectively. Two-thirds of the total energy cost of the meat processing industry is equivalent to the cost of electricity (HTC, 2009). According to the FAO meat price index, the average international meat price in 2019 is 175.7, which is 9.4 points (5.6%) higher than that in 2018 and 2.3% lower than that in 2018. Production of other meats, especially poultry, is on the rise, while import demand is surging. Increasing meat production and exports are the response measures of many meat-producing countries, but the total global exports are still far below the level needed to fill the deficit, leading to the rising trend of international meat prices (FAO, 2020b).

Society, manufacturing and food processing industries are facing technological and economic changes. Therefore, the whole food supply chain has been greatly affected. In order to meet the needs of consumers' healthy lifestyles, enterprises attach great importance to food. The necessity of survival in the fierce competition is to introduce market innovation into the food industry to further update, so large-scale research and exchanges were held. Many achievements have been made in this regard. The protection of agriculture is guaranteed by the international trade system under the GATT. In order to achieve the goal that a country's agriculture will continue to be the top priority sector of all countries and people, which can promote the creation of healthy societies, meet food needs and provide nutritional security. Agriculture not only contributes a lot to GDP, but also provides food, raw materials and fiber for industry (Galanakis, 2018).

People's food is based on products such as milk, cheese and yogurt, which are widely consumed all over the world. Milk is the world's largest consumer, but the milk of sheep, camel, buffalo, goat and other mammals is also being consumed. In November 2020, the United Nations Food and Agriculture Organization (FAO) dairy price index continued the upward trend in recent months, with an average of 105.3 points, up 0.9 points (0.9%) on a month on month basis, approaching the highest point in 18 months. The recent rise is mainly due to the steady growth of global import demand, which is due to the rising prices of butter and cheese, as well as the surge of retail sales in Europe. The milk market is disturbed by Covid-19. On the contrary, it is expected that the global milk production will increase by 1.4% year-on-year to 860 million tons in 2020 (FAO, 2020c). Dairy products are important sources of protein and calcium, and play an important role in nutritional diet. Dairy industry has become one of the important sub industries of animal husbandry (Zocca et al., 2018). Many studies emphasize that dairy consumption in Europe, the United States and other parts of the world is the rate of energy consumption. The cheese manufacturing industry, for example, can be used to evaluate and measure the energy performance of the food industry (Nunes et al., 2014; 2015; 2016). The consumption of dairy products in China is on the contrary, and the per capita consumption of dairy products is lower in areas with higher population density (Silva et al., 2016). The new trend of the times will become an industry-led information age. To provide a series of safe, healthy, nutritious, affordable and sustainable food for consumers and society is the main goal of agricultural products processing industry under the premise of introducing new technology and maintaining competitiveness. On the whole, a processing plant has the characteristics of limited resource utilization and serious corruption, so it is called agricultural products processing industry, so its use should be as efficient as possible. In general, due to the importance of agricultural products in the human food chain, the industry has changed agricultural products, including not only human food, but also animal feed. Due to the positioning between agriculture and consumer market, the agricultural products industry has its independent characteristics, and the construction and development of sensitive raw material behavior and market organization (Zocca et al., 2018).

4. Agricultural trade

Agricultural trade has been covered by the general agreement on trade and tariffs (GATT), signed in 1946. In the bilateral aid, the share of production sectors including agriculture, mining, industry, tourism and trade policy continued to increase. (WTO, 2000). GATT and World Trade Organization (WTO) have promoted trade and innovation by reducing tariffs many times, combining discipline with basic principles, and reserving policy space to deal with important social issues. WTO disciplines will continue to promote trade and innovation in the digital world. In addition, the multilateral trading system provides certainty, promotes cooperation, and is flexible in dealing with new problems (WTO, 2020b).

In the world agricultural development report, the main driving forces for growth in developing countries are divided into three categories, including agricultural economy (agriculture accounts for about 30% of GDP), transitional economy (agriculture accounts for about 19% of GDP) and urbanization economy (agriculture accounts for about 7% of GDP). In the transition and urbanization economy, industry and service industry are considered as the main sources of economic growth. Only in the first agricultural based economies, mainly in Africa, should focus on agriculture as the main driver of growth. In fact, agricultural and non-agricultural sectors are closely related in terms of inter-sectoral demand (including intermediate input). Therefore, the focus of investment may need to take into account the indirect role of the agricultural sector in stimulating rural income growth (WTO, 2008). More and more attention has been paid to the distribution of trade income, so the dominant paradigm of international trade has been under considerable pressure (Viju-Miljusevic, 2019).

Agricultural trade is of great significance to the development of national economy, especially to employment and food security. It is controversial to bring agriculture into the rules of international trade. The export of agricultural products is very important, and the import is equally important to the food security of consumers in net food importing countries. Each country has the right to produce enough food, protect vulnerable farmers, and develop unique agricultural policies. So far, the approach taken by the international trading system has been to enhance the flexibility of multilateral and preferential trade agreements. Market access, domestic support and export subsidies are the three major agricultural agreements. Export subsidies will increase exports, raise domestic prices and reduce foreign prices. A price wedge equal to the subsidy value will be formed between the foreign price and the domestic price of the product. These measures need to follow standards to limit their impact on trade and production (Peters et al., 2013).

Agricultural capital refers to the value of farmland, including agricultural machinery and equipment, buildings, livestock and poultry for agricultural operation. However, it does not include the value of existing agricultural inputs such as fertilizers, crops or seeds in the field or stored. Farmland includes all land owned as part of its business activities, including hay grazing or pasture, swamps, buildings and barns, summer fallow, woodland and arable land. The person responsible for the day-to-day management and decision-making of a farm or agricultural operation is referred to as "farm operator", and each farm reports up to three farm operators (Cook, 2018).

A major challenge for low-income countries is to design and expand alternatives to social protection for workers in the informal economy (Jansen and Lee, 2007). Formal employment is more important in middle-income countries, and there is often more scope for social protection for workers adversely affected by trade and related economic reforms. The time required to import and export goods is an important trade barrier. Trade liberalization provides business opportunities for companies that are able to export, and offers consumers access to cheaper and different goods through imports. However, these imports may compete with local production, and the local producers concerned may be under new competitive pressure. New export opportunities and increased competition from imports will lead to the expansion of some activities and the reduction of others (WTO, 2008).

In developing countries, the supply of exports and employment opportunities has also accelerated the pressure on agricultural trade. Agricultural trade liberalization alone cannot create an employment miracle. Similarly, agricultural trade liberalization should not be expected to have a significant negative impact on employment, but these successful strategies are based on agricultural trade. Many agricultural workers need to adjust these plans more reasonably to reduce their social security burden. Trade liberalization is to eliminate or reduce the restrictions or barriers to tariffs (such as tariffs and surcharges) and non-tariff barriers (such as license rules and quotas) on the free exchange of goods between countries. Trade liberalization can lead some developing countries to further increase the specialization of agricultural production. The migration from rural to urban is the concentrated embodiment of a country's population. Measures to promote urban integration may have a significant impact because trade reforms trigger or exacerbate such migration. In order to promote food security for new urban residents, more information and facilities on housing or employment opportunities can be provided, or suburban agriculture can be supported, including planting crops and raising livestock around the suburbs. Although the poverty rate of rural workers is very high and it may cause great difficulties to move from one job to another or from one place to another, reducing this difficulty is a lofty goal and may help to improve economic efficiency (Cheong et al., 2013). The e-commerce and law reform program of the United Nations Conference on Trade and Development (UNCTAD) provides an opportunity for developing countries to conduct expert reviews of e-commerce legislation and to provide expert advice to policy-makers on effective e-commerce laws. The areas covered by the scheme include consumer protection, cybercrime, data protection and privacy, intellectual property and electronic signature (WTO, 2020b). Where does the food come from? If past trends continue, expanding trade will not be the answer. Since the reform of agricultural trade, the world grain output has more than doubled, and the world grain trade has also doubled. Therefore, the share of food consumption in world trade remains around 10%. This shows that 90% of the world's food production is consumed on average in the producing countries. If this trend continues, it is clear that most of the growth in food production must come from the production systems of countries where new populations live (McCalla, 2001).

Sustained agricultural investment will inevitably have positive side effects on the agricultural industry, as Z manufacturing (such as food and beverage) shows. The agricultural sector is likely to play a greater role in the global economic integration. In populous developing economies such as India and China, per capita income and food demand are also growing, indicating that the agricultural sector has considerable expansion potential. The agricultural sector has always been the foundation and component of the world economy and development. It plays a role in providing food and raw materials to the domestic market, absorbing domestic labor and capital, and generating export income. It also supports manufacturing and services. However, when formulating effective and up-to-date policies on agricultural trade and sector linkages, the impact of trade on agricultural value-added and the spillover effects of agriculture on the world economy and other sectors deserve further analysis. The conclusion is that policy-makers need to seriously consider new agricultural policy initiatives to bring the agricultural sector closer to other sectors such as tourism services (Gani and Scrimgeour, 2019). In fact, in many of the world's economies, the trade and agricultural sectors have been the main areas of research. If a country wants to be strong, it must rely on itself to develop an agricultural economy and ensure ecological and nutritional security.

5. Conclusion

In order to promote “modern agriculture” and overcome “challenges”, it is necessary to further strengthen agricultural scientific research. On the road of common development of agriculture, industry and commerce, we should first prevent and avoid food shortages, disasters and conflicts. Secondly, we need to find new methods of international trade competitiveness and obtain the growth of agricultural production level; at the same time, we need to realize sustainable development.

We should learn from the past experience that no matter how many problems there are, solutions should be given priority. The problems are not insurmountable but it uses modern agricultural methods to get through them. A comprehensive survey of the challenges facing today’s agriculture, such as Covid-19, population growth, changes in eating habits, destruction of seed resources, shortage of water resources, air pollution, climate change and changes in food prices, shows that it is the common responsibility of all countries to invest the most in solving these problems. In addition, floods and droughts continue to affect the growing season of crops, limiting water supply, increasing the growth of weeds, pests and fungi, and reducing crop yields.

With the simultaneous development of food security and industry and commerce, we need to completely change all social production and consumption patterns. To protect and strengthen the reserve of natural resources, maintain and improve the quality and efficiency of sustainable grain and modern agricultural production through the innovation and competitiveness of agricultural modernization system, is one of the main focuses of modern agricultural development policy. Although many studies emphasize the potential of traditional agriculture, the machinery and technical facilities in the agricultural sector, as well as the relationship between supply and demand, are changing rapidly.

Today, for example, genetically modified plants have reached a huge commercial scale. The technology of artificial meat is more and more popular. I hope this product can be launched as soon as possible. The greatest hope for meeting the challenge of sustainable agricultural development lies in the ongoing innovation process, which uses modern genes and information technology to improve agricultural productivity, while balancing the economic, health, environmental and social outcomes related to agriculture and food systems. Great changes are taking place in the face of agricultural modernization. The innovation practice of traditional industries is speeding up the improvement of new products. Especially in urban areas, people are more and more interested in innovative food and natural products. They are opening up new markets and opportunities.

Agriculture is one of the oldest commercial forms of industrialization. At the end of the 20th century, from the beginning of “The Green Industrial Revolution”, agricultural production based on cultivated land, planting plants and raising animals has become an important part of national economy in developing countries. Since then, agricultural industrialization has continued to grow, including the production, supply, sales and export of agricultural products. The industry has become an important industry in India, Africa and other ecological countries. Ecological country’s goods and services, laws, guidelines and policies are environmentally friendly, also known as eco-friendly, nature friendly, or green, which means that the practice is sustainable, reducing or minimizing the damage to the ecosystem and the environment. Being eco-friendly helps to save water, energy and other resources and prevent air, water and land pollution. Environmental performance index (EPI) is a method to measure a country’s policy environmental performance.

Human safety is directly proportional to food safety. As a human standard, it needs to be divided into material and spiritual supplements. The development of an economy is in direct proportion to the support of economic investment. The only barrier to economic integration is the existence of interest. The development of an agricultural economy is also the development of trade and industry.

If Covid-19 prevents low-income countries from getting rid of poverty and further promotes agricultural modernization, otherwise population growth may be higher than previously predicted, which will bring greater pressure on food security, trade and industry.

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Review article

The new era in office-based facial rejuvenation: Promising technology of silicone threads

Naci Celik*[1,2]

[1] Istanbul Okan University, Faculty of Applied Sciences, Department of Gastronomy, 34959, Istanbul, Turkey
[2] Plastic and Reconstructive Surgeon, Private Practice in Dr. Naci Celik's Clinic, 34367, Sisli, Istanbul, Turkey

Abstract

Aging is unpreventable, although its symptoms vary a lot among individuals because of the genetic determinants and one's life habits. Sun exposure, bad habits like excessive alcohol consumption, and smoking accelerate the aging process and urge people to seek for a solution to reverse the changes, especially for the most prominent part of our body, the face. Unfortunately, there is no one simple solution for that, and it includes a bunch of surgical and non-surgical interventions. Relatively simple methods have fewer risks, but the reversal effect is also minor. This includes neurotoxin and filler injections as well as energy-based devices. More competent surgical options, alas, come with a long and difficult recovery period and diverse, sometimes inevitable, complications. Most of the time, people are scared of the surgery and accept less invasive methods. Among these, thread lift is perceived as the missing link between the surgery and non-invasive methods. Unfortunately, up to recent years, the results of threads have not been promising, and they also have many complications. A new type of thread originated in France, made of silicone and polyester, gives promising results. This paper reviews the history and specifications of the threads and tries to explain the logic of their use in facial rejuvenation.

Keywords: *Facelift; infinite-thread®; silicone threads; spring thread®; threadlift*

1. Introduction

Aging is a degenerative process and a complex biological phenomenon caused by intrinsic and extrinsic factors (Charles-de-Sá et al., 2018). Intrinsic aging is largely genetically determined and affects the skin through a slow and partly reversible degeneration of connective tissue (Uitto et al., 1986). On the other hand, extrinsic aging, primarily ultraviolet radiation, results in premature aging even in young individuals (Scharffetter-Kochanek et al., 2000). The neck and face are sun-exposed areas like hands. These areas are under an overlapping influence of intrinsic and extrinsic factors, which produces a more complex and faster aging process (Fisher et al., 2002). The symptoms of aging can be concealed in some areas, thanks to advanced surgical techniques (Rodriquez-Bruno and Papel, 2011), amazing hyaluronic and non-hyaluronic acid fillers (Tan and Kontis, 2015), neurotoxin treatments (Raspaldo, 2011), energy-based devices (DiBernardo et al., 2016), and evolving thread technologies (Rezaee Khiabanloo et al., 2019).

Aging also results in a deficiency of facial fat compartments (Cenkeri et al., 2020), which can easily be reversed with fat injections (Obagi and Willis, 2018) or facial fillers (Philipp-Dormston et al., 2020). On the other hand, neither fillers nor the energy-based devices cannot be used successfully to lift the descended parts of the face; however, they are great adjuncts following facelift to obtain a better aesthetic outcome (Hammoudeh and Stevens, 2019).

Surgery is usually the preferred choice of treatment for optimal results in face and neck rejuvenation. There are several surgical face and neck lift methods (Beaty, 2014). Although

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[*] Corresponding author.
E-mail address: drnacicelik@gmail.com (N. Celik).
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surgery's success is not deniable in this area, there are particularly challenging cases either due to anatomical variations or psychological expectations based on the individual's perception of the problem and motivation for change. A thorough patient evaluation and counseling are mandatory before considering any surgical approach in the neck and face, and modifications of the surgical approach are needed, if necessary (Smith and Papel, 2018). On the other hand, surgery alone may fail to meet some neck problems, such as superficial aging changes, as it addresses excision only. Chromophore-based pathologies, vascular changes, epidermal and dermal nonchromophore-based lesions are among these problems (Mulholland, 2014). It is also important to individualize the relationship between the lower face, jawline, and neck (Celik, 2020a). As people age, some of the lower facial tissues descend beyond the jawline, which makes the correction of facial tissues crucial to accomplish a substantial improvement in the aesthetics of the neck (Rohrich et al., 2006). In brief, good results with face and neck rejuvenation can be achieved if it is combined with lower face and jawline procedures, filler injections, and the inclusion of energy-based therapies (Celik, 2020b).

Nonetheless, there are three important reasons for writing this paper;

1. Surgery offers its advantages together with its complications, like a longer recovery period and inevitable scars.
2. Not every doctor can perform this kind of surgery. This includes inexperienced plastic surgeons.
3. Not every patient is fond of surgery, and they are looking for the so-called “minimally invasive procedures”.

Both patients and the doctors need a bridge between the surgery and non-surgical rejuvenation (Celik, 2020c). For more than two decades, thread lift methods tried to fill this gap, however in vain, most of the time.

2. Historical background and classification

2.1. Non-absorbable polypropylene threads

Thread lifting has come to its current place after a long journey (Savoia et al., 2014), which began in the nineties with non-absorbable polypropylene threads (Sulamanidze et al., 2002). The doctors' early excitement created popular office-based procedures called a lunch-time facelift by media (Atiyeh et al., 2010). However, they fell out of favor because of high complication rates and fast temporary results (Lycka et al., 2004). Most of the complications encountered were due to non-absorbable suture material (Silva-Siwady et al., 2005). These types of complications necessitated surgical interventions to solve a problem of non-surgical intervention. Complications and lack of a good long-term effect of the threads were the most common reasons for the technique's abandonment (Rachel et al., 2010).

2.2. Absorbable threads

With the introduction of mixed absorbable and non-absorbable threads and pure absorbable threads, thread lifting has again gained attention. Several manufacturing companies have produced diverse types of threads. Silhouette Soft® suture (Silhouette Soft®, Sinclair Pharma GmbH, Irvine USA) was made of poly-L-lactic acid (PLLA) and consisted of multiple cones that were made of polylactide/glycolide copolymer (PLGA). This thread was an absorbable one, and it was different from the other threads of the same company. Silhouette Lift® was composed of non-absorbable polypropylene suture and absorbable PLGA cones. Silhouette Instalift® contained only PLGA both in sutures and cones. PLLA and PLGA are biodegradable polymers, and PLLA is a well-known biomedical device for over four decades and has been used as absorbable plates, screws, and suture materials. PLLA triggers a foreign body reaction when implanted into the tissue. This reaction generates a cellular inflammatory response, which leads to the formation of vascularized, connective tissue (neocollagenesis) (Bohnert et al., 2019). This neocollagenesis, when coupled with repositioning by the sutures' cones, makes these kinds of suspension sutures a valuable tool for facial rejuvenation (Goldberg, 2020).

Another kind of absorbable thread that is used for facial rejuvenation is polydioxanone (PDO). Polydioxanone sutures were in plastic surgery as intradermal sutures since the '80s (Chusak and Dibbell, 1983). Years later, originated in Asia, PDO threads became available for facial rejuvenation around 2015 (Suh et al., 2015). PDO is a synthetic polymer and absorbed from the body by hydrolyzing in 6 months. The addition of barbs to PDO tries to increase the load-bearing ability when used as suspension sutures. However, the aim is to lift the ptotic facial tissues, polydioxanone could not achieve this goal, and it is often used as "solid fillers" to treat deep static wrinkles on the face (Kang et al., 2019). Polydioxanone fills the wrinkle first by its volume and later by the mild local inflammatory reaction, which results in lymphocytic infiltration and subsequent fibrosis. Although there are many variations, polydioxanone threads for facial rejuvenation can be classified roughly into three different types:

1. Mono PDO thread: non-barbed and thin (0.07-0.15mm) monofilament thread.
2. Spring or twin thread: Braided 2 monofilament PDOs or twined single monofilament. It is more tensile than mono PDO.
3. COG PDO thread: This one has barbs and creates a lifting effect when pulled. The cogs were shown to induce a fibrotic reaction four weeks after the insertion (Jang, 2005). Depending on the direction of the barbs, they can be categorized as:
   a) Unidirectional.
   b) Bidirectional.
   c) Multidirectional.

Although absorbable threads were relatively well-accepted by dermatologists and aesthetic practitioners, plastic surgeons were distant from these kinds of procedures since the beginning. Many cosmetic companies produce their own so-called thread-lifting sutures, and these companies have spent a great deal of marketing budget for the training of physicians and non-physicians. In combination with enthusiastic cosmetic professionals, these commercial encouragements are mainly responsible for the extensive spread of this alleged "minimally invasive" lifting procedure. On the other hand, even the most optimistic non-surgeon doctors emphasized that thread lifting is neither an alternative to surgery nor magic per se, but it could have good results for rejuvenation and skin tightening, especially when combined with other tools of rejuvenation (Ali, 2018). Although the facelift effect seems very subtle, the complications of absorbable sutures are regarded as minimal or moderate without permanent sequela (Sarigul Guduk and Karaca, 2018). In another study, despite the results seemed to be good, the authors concluded that, because of the high complication rates of the PDO threads, short-lived benefits, and similar downtime and costs, traditional facelifting was to be preferred (Bertossi et al., 2019).

Most of the studies published show only a limited effect and longevity. A recent review of thread-lift sutures (Gulbitti et al., 2018) concludes that the use of threads seems to be promising when they are used in combination with an open procedure.

2.3. Silicone threads

Being a skeptic plastic surgeon, the author of this publication refused to use threads until 2016, when non-absorbable silicone threads were introduced in the Turkish market (Spring Thread®, 1st SurgiConcept 96 Rue de Pont, Rompu 59200 Tourcoing, France). Silicone threads do not rely on fibrosis and remodeling and subsequent skin tightening for lifting effect as absorbable threads do. Instead, their mechanism is simpler and more rational for a plastic surgeon; the lift effect is created by the upright movement of tissues, thanks to the barbs. Although the mechanism and logic were similar to primitive non-absorbable sutures, the technology and the material used are different. Silicone thread is a biocompatible composite material. The outer part is medical grade silicone, which envelopes the polyester inner part. It is elastic and can be elongated by 20%. This elasticity provides a spring effect that compensates for the creep of classic threads. The author used this thread alone and in combination with surgery (Celik, 2020d). Although it is beyond this paper's scope, the main advantages and disadvantages of Spring Thread® should be addressed concisely.

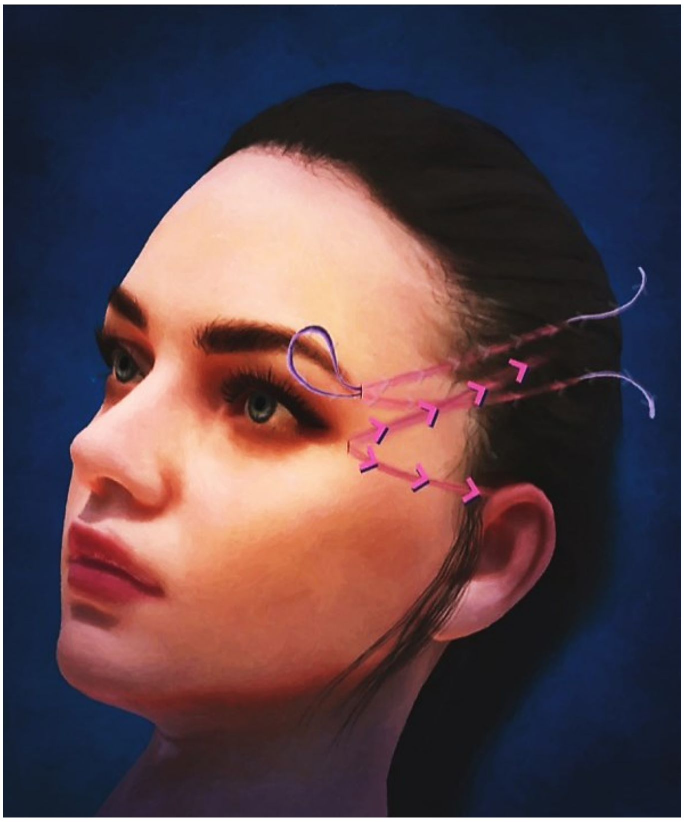

Fig. 1. Drawing of thread placement for browlift and canthopexy.

The usual thread reactions like extrusion or granuloma formation encountered in the polypropylene threads were not seen in silicone threads. The lifting effect was more powerful than the other barbed sutures that the author used before. On the other hand, slipping of the silicone coating from the polyester core during the thread's implantation was not unusual, necessitating another box of thread. According to the author, another problem was the elasticity of the silicone. However, in the beginning, it was presented as one of the advantages of this new type of thread; by the time, it was realized that the elasticity was one of the disadvantages of this thread. Briefly, elasticity causes shorter thread length and less amount of barbes inside the tissue. The thread shortens back to its unaltered length after some time, and the pull effect decreases. Later in 2019, After 2 years of experience with more than 200 patients, the author's perception about the threads was that they could not replace open-type face and neck lift surgeries but can be applied to many patients who are avoiding the complications of open surgery and seeking for some effect of a facelift for a few years at least. For this purpose, the author designed a new way of eyebrow lift and canthopexy instead of an endoscopic temporal lift (Fig. 1).

In 2019, the author started to use non-elastic silicone threads (Infinite-Thread®, Thread & Lift Laboratory, Brussels, Belgium). This one, just like the other silicone thread, is made in France, but the headquarter is in Brussels. After using Infinite-Thread® for a minimally invasive facelift, the author realized that this thread is a powerful game-changer. It has some unique properties which are different from the previous threads. It also has a polyester core coated with silicone. There are 4 cogs in every 1.5 cm. Each series of cogs is offset at 45 degrees, and it creates an "8 axis" hooking. The diameter is 1.4 mm from cog to cog, but the cylinder of silicone is 0.5 mm. Cogs have a unique design that prevents them from turning and slipping from the

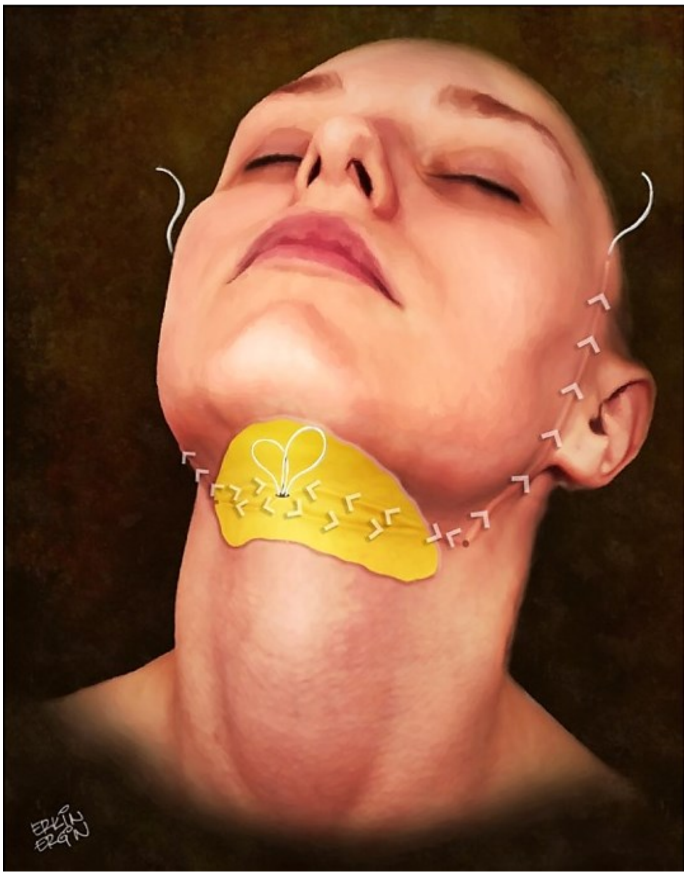

Fig. 2. Schematic representation of neck lift with silicone thread.

tissue thanks to their conical shape, which reinforces the cog at the base. Cogs have rounded tips that prevent the cheese-wire effect (a common problem of early polypropylene threads). Because of this new thread's powerful lift effect, a neck lift is also possible (Fig. 2). Infinite-Thread® is not elastic deliberately (does not elongate) but flexible.

There are not a lot of scientific articles about the results of silicone threads for facial rejuvenation. Although according to the author of this paper, non-elastic silicone thread seems promising, we need more studies to see their long-term effects and their capacity to lift the face.

3. Discussion

The minimal invasive facial rejuvenation concept is tempting for patients. It has gained high popularity among physicians and patients looking for an easy facial lifting method and skin rejuvenation. Although the plastic surgeons' curiosity has withered because of the fast-temporary effect and a bunch of complications of the threads, the patients have never lost interest, thanks to cosmetic doctors who offer the procedure (Celik and Gok, 2020). On the other hand, the literature review shows no evidence to support the threads' effectiveness unless studies are supported by industry.

We encounter many rumors of perfect lunch-time facelifts, websites promising perfect results, media interviews, and case reports. However, we do not come across detailed scientific papers about the results, long-term follow-ups, and other types of information that we used to see in scientific publications on other subjects. That is why plastic surgeons have lost their trust in threads, no matter what the material or the technique is. There are rare but promising publications about thread use in open surgeries published by surgeons (Matarasso, 2013; O'Connell, 2015). A combination of threads with other methods of facial rejuvenation also seems promising (Celik, 2020e). Nevertheless, the success of these encouraging results is not only because of the threads but because of the other techniques used with the threads, and they are far away from being a minimally invasive office procedure. On the other hand, recent developments in thread technology reveal a new era in facial rejuvenation. Under local anesthesia, office procedures may have a similar rejuvenating effect to those of open surgeries with minimal downtime and less risk of serious complications with these new silicone threads. This is especially very important for plastic surgeons who are strictly adhered to conventional methods. There is a publication from 2008 by D'Amico et al. which had seen the future (that future is our today now) and warned the plastic surgeons about the imminent danger of negligence. Their paper clearly shows the trend of the consumers (patients), plastic surgeons and non-plastic surgeon core providers (dermatologists, ENT specialists), and non-core providers (the rest of all cosmetic procedure providers) at that time. One vital message that can be learned from this study is that the patients would choose plastic surgeons to perform more invasive procedures (90%), but the percentage decreases rapidly when it comes to less invasive (40%) and the least invasive procedures (15%). Another important note is that of consumers who had had a positive experience with a non-plastic surgeon for a non-invasive procedure, 47 percent said that the same provider would be their first choice for an invasive procedure (D'Amico et al., 2008). This means that should the plastic surgeons keep the distance to these minimally invasive methods, their number of surgical cases may drop over time.

4. Conclusion

Thread lifting is not a complication-free nor a minimal invasive lunch-time beautification procedure. Quite the contrary, it is not different from surgery in terms of complications, technical demands, and effects. Then why should we choose thread lift instead of surgery? The author of this paper thinks that we should not adhere to one of them. We should choose the right technique for the right patient. The decision should be made by a mutual agreement between the doctor and the patient. We should quit promoting surgery while vilifying thread lifts. One of our latest unpublished studies shows that 75% of patients who ask for thread lift and are refused by the doctor and offered surgery would find another doctor who would do the thread lift. More than half of the remaining 25% forgoes any procedure. Plastic surgeons should overcome their prejudice and give another chance to evolving thread technology.

Conflict of interest: The author declares that they have no conflict of interests.

Informed consent: This manuscript did not involve human or animal participants; therefore informed consent was not collected.

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