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Wildlife Mortality on Single Track Bhalwal Road, Sargodha, Pakistan

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ABSTRACT: *Wild animals are very important for our ecosystem maintenance. In the last few decades, the rate of animal's accident on roads has increased due to heavy traffic. This research was carried out to find the reasons for road mortality of animals on Bhalwal Road, Sargodha, Punjab, Pakistan. It was noticed various animals like rats, porcupines, lizards, snakes, turtles and jackals lived in crops and trees that were found around the road and they were killed by road traffic while crossing the roads. It was seen that almost 80 to 90 % of road mortality was due to over speed driving. Moreover, the travellers were not aware of the importance of these common wildlife, so they did not bother the animals moving on the road. It was observed that most of the animals that killed on roads belonged to reptiles (Monitor Lizards'), amphibians (Frogs and Tortoise) birds (crow) and mammals (Jackals, mouse, dogs, cats and porcupines). It was concluded in the study that there is a need to highlight the importance of wild animals for the locals, travellers and also for the stakeholders that start work on mitigation strategies that help to conserve these wild animals.*

Keyword: *Bhalwal, Sargodha, Mortality, Wildlife, Ecosystem, Mammals*

INTRODUCTION

Isolation of habitat and restricted movements of common wildlife affect the animals that living around the roads. Roads playing a vital role for animals that living on the earth. Most of the species which belonged to class reptiles and amphibians are killed by crossing roads in search of food, habitat or to meet other animal. Some species used roads to get heat, nesting, food or various other purposes (Fahrig and Rytwinski, 2009).

Most of work started in Canada by Canadian Wildlife Service (CWS) in 1970 and 1980 and later in 1992 to 1993 and reported that animals which living in water and land mostly affected by road accidents (Clevenge et al., 1994).

Traffic can affect animal populations in two ways: firstly to kill the animals and secondarily, by dividing the habitat of population into fragments (Gregory et al., 2021). Roads are very important tool for movement of animals from one place to another however, they can create a lot of chances to increase the mortality rate of animals that lived around the roads (Oddone and Nkomo, 2021).

Many other things can affect the mortality rate on the roads such as traffic rush, location of road and climatic conditions of that area (Clevenger et al., 2009). Biologists and zoologists have work on the new and safe movements facilities for the animals that live across the road (Forman et al., 2003). Some solutions such as fencing and channelling across the road can reduce the road mortality due to some new and safe ways of road crossing (Clevenger et al., 2009).

Traffic load on roads causes different types of effects on nature either directly or indirectly such as climatic changes and influences on natural environment. A lot of work is being done by many researchers and road management authorities on different aspects of roads to reduce the ecological effects of wild life on nature (Coffin, 2007).

Wild life conservation possibly driven by different ways such as tunnels constructions, overpasses, fencing around the roads and prevention of road mortality of common wildlife (Bennett, 2017). That effort is a remarkable work to decreased mortality rate of common wildlife in different countries such as America and Canada (Blaustein et al., 1994).

Various ecological changes such as food intake by man, cosmic rays, and acidic concentration of water leads to the transfer of predator species from one area to another that leads towards the decrease in number of wildlife species in certain area. Hundreds of masses of creatures every year murdered by road traffic flow (Rytwinski, 2016).

Because of animals shifting from their habitat to another area they need to cross the road that leads towards road collisions and animal killing. Expansion of roads to make them wide also causes the reduction of wild life as their ecological systems are disturbed (Eigenbrod et al., 2009). Night vision animals have more mortality rate on roads (Mazerolle, 2004). Across the protected area the spreading of the roads has effects on incensement of the large animals (Grosman et al., 2009). In India and mostly many parts of Asia having different kind of religious or sacred places for the completion of their religious worships and gathering all around the nearest areas that consumes the area of flora and fauna near mountains. After creating that kind of situations mostly large animals spare that place but small animals not move and became the victims. From last 50 years the volume of the traffic on roads in Asia has increased (Seshadri et al., 2009). As in rest of the world, there are also certain issues concerning human wildlife conflicts in Pakistan but less documentation available yet (Ahmed et al., 2016). The studies from Pakistan has focused more on the northern regions of the country (Younas et al., 2018), where there is large number of wildlife but area is less human-dominated comparatively (Rehman and Khattak, 2020).

This study was conducted in order to know the mortality rate of various animals, birds, reptiles and amphibians on Bhalwal road, Sargodha and to find out their movement from one habitat to another for their survival.

MATERIAL and METHODS

Study Area

The present study was carried out from March to June 2018 to study the local wild animals such as jackals, dogs, reptiles, amphibians and other animals that live across the road at different sites. The selected sites were Site # 1; Bhalwal to 10NB(2KM), Site # 2; 10NB to Chak 22 NB (1KM), Site# 3; 22 NB to 23 NB(3KM), Site # 4; 23 NB to Ajnala lok (7KM), Site # 5; Ajnala lok to Sargodha (8KM). These sites were selected as these are the connecting

roads among different polluted areas and have running traffic load.

Study Visit Timings

Monthly two visits were done of each place for data collection. Data of killed animals was recorded from these sites. The method that we followed was direct field observation and counting of killed animals and traffic numbers. Beebee. (2013). First step was to tour the road twice a month and capture the pictures of killed animals on Bhalwal road. Second step involved collecting data of traffic vehicles average number in a month.

Road Kill Count

Number of killed animals was recorded during this study. The data was collected by road tours on regular intervals from April to August. We could only count the number but sampling was difficult due to the condition of animals and age was also unidentifiable. Simple counting method was used for this purpose.

RESULTS

A total number of 242 killed animals were calculated during the study period. Mostly the night vision animals were killed by traffic due to heay road traffic such as trucks, loaders and long passenger buses. The number of jackals was high as compared to other animals on that road. It was perceived the carnage of these faunas on Bhalwal road was due to some high-speed heavy traffic on imminent time. The speed limit was above 100km/h without following the allowed limit rules. That was alarming speed on local road across the rural areas. The night idealistic animals used their night strategies to pass the road and mostly strike with high speed vehicles. In these animals mostly the idlest animals were maximum. The load on that road mostly was between 7pm to 8:30 am. During this duration, 34 lizards were found dead from April to August 2018. Due to many reasons' reptiles were killed by the road traffic such as slow speed (Fig. 1).

Fig. 1. Lizard killed on Bhalwal Road, Sargodha, Pakistan

Porcupines were killed by traffic due to nocturnal way to find out the food and its habitats were seen around the roads (Fig. 2).

Fig. 2. Parcoupines killed on Bhalwal Road. Sargodha, Pakistan

Due to unavailability of large water bodies around the road the number of amphibians was less as compared to other animals. Two frogs and one

tortoise were also noticed killed in study time period (Fig. 3).

Fig. 3. Tortoise killed on Bhalwal Road. Sargodha, Pakistan.

Although some seasonal pond had formed by rain. N=145 was the greatest number of killed mammals on the Bhalwal road as compared to other animals in this study duration. Mouse was the smallest animal noticed on Bhalwal road, Sargodha, Punjab, Pakistan (Fig. 4).

Fig. 4. Jackal killed on Bhalwal Road. Sargodha, Pakistan

A jackal was also noticed killed on road and has shown in (Fig. 5).

Fig. 5. Mouse killed on Bhalwal Road, Sargodha, Pakistan

It was very important thing on Bhalwal road the mouse or small animals just like that were very rare. This matter might be a low rate of these animals' existence, low numbers of their habitats, low number of their life helping factors. Their counting of killing animals were so difficult due to very small body size as compared to the busses or road traffic. Their body was consumed by microorganisms with very fast and very quick. Five-month data were collected on the basis of road mortality of common wild animals that lived across the Sargodha to Bhalwal road. In April the area of that Bhawal road was going to be tough because of temperature. April having some sort of rains but not as the month of June, July and August. Counted numbers of the dead animals in the month of the April mostly observed the species were killed on the night time.

The monitor lizards mostly killed on the morning times. Snakes were not observed in the month of April. Jungle crow were observed during afternoon and may be due to cross the road but having a high weight of that birds make a collision with large vehicles such as trucks, 12 wheelers or long passengers' buses. No amphibians were observed

during this month. These ponds may be produced by the rains on that area in the months of May, June, July and August. Mammals observed all the months of years due to large numbers of these animals having their habitats. Results were analysed by the statistical analysis and used the two way ANOVA. The p value was <0.05.

In month of May, the temperature of Punjab area is mostly 38 to 45 ℃. Due to rain some pounds were formed automatically across the road sides of Bhalwal road. When we saw the total number of killed animals in the month of May, it was n=51. Reptiles were observed but no snake killed. Amphibians have nothing because of water availability in almost zero. In view of monitor lizard mostly observed the young and matured monitor lizards. It is also observed that the speed of that animals and its weight takes it on 90% chances to killed through traffic when it passes through the road. It had both side habitat. Snakes are observed by some native presents. But there was no road mortality of snakes on that month. it was due to the speed and sensor ability of stimulus acceptance on different body parts. Among birds only jungle crows were observed. Jungle crow has a large weight and when it crossed roads the collision of that birds was almost possible due to low fly. Mammals have large numbers of road mortality due to large size large numbers and night visionary behaviours. It is also observed that the amphibians also seen in that month.

In the month of June, the total number of murdered faunas was n=29. Number of killed reptiles was 5 but only observed 4 monitor lizards. There was a snake killed in that month of June. Amphibians were less but found 1 killed tortoise and 2 frogs because of water availability. We mostly observed the young and matured monitor

lizards were killed in that month. It was also observed that the speed of that animals and its mass receipts it on 90% risks to murder through traffic when it passes through the road. It has both sides habitats. When we saw the birds, jungle crow was observed. Jungle crow have a large bulk and when it cross roads the crash of that birds is almost conceivable due to squat fly. Mammals had large numbers of road mortality due to large size large figures and night idealistic manners. The killing number of frog and tortoise in that month indicates a pond well contacted due to increased raining. Total number of killing animals in June was 29 that was less as compared to May. It was a difference due to different numbers of crossings on that road on different time framework of time.

An overall five months data of killed animals were represented in (Fig. 6)

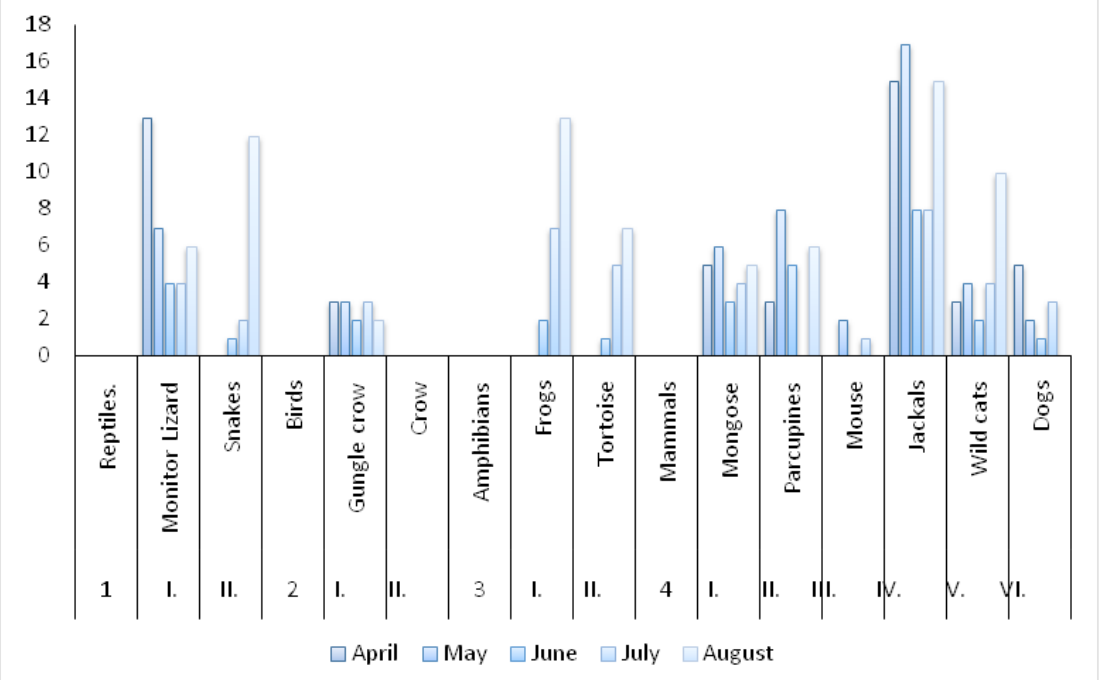

Fig. 6. A record of total number of killed animals in 5 months (April-August)

DISCUSSION

This study helped us to identify the number of animals from different species that are being killed by road accidents every day. Different reasons are responsible for this killing of animals as they cross the road. The study was conducted on Bhalwal road, Sargodha, Pakistan. During observation it was noted that the total killed numbers of animals was 242. In five months observations, the highest mortality rate was obtained for mammals that was 47.9 % followed by amphibians that was 26.31 % and the 24.23 % for reptiles. This was also supported by previous study in Pakistan where the highest rate of killing was observed for mammals (Akrim et al., 2019). Maximum number of jackals indicate that they came there for feeding on other dead animal carcasses and got killed by themselves due to poor visibility of drivers at night.

The results of birds killing revealed that only thirteen birds were found dead. So, the death rate was 2.63 % of the total killed animals. The bird's mortality on Bhalwal road when compared to the road mortality of birds in previous study gives the same results and the reason for less number is because of their flight behaviour. birds are mostly killed by electricity pools around the roads on height (Akrim et al., 2019).

In our research work, the number of killed animals increased in the amphibian's phylum that was 26.31 %. Due to pond constructions in the rainy season some ponds formed due to lower surface across the road on Bhalwal road Sargodha. Less speed, less development of behaviours learning and some other crossing reasons made them contact with traffic and collisions occurred. But in April and May have no road mortality due to almost zero water or pond availability.

In our research we identified that reptiles accounted for 24.23 % of total road killed animals that was third highest number on Bhalwal road. Reptiles have less speed due to large body size such as monitor lizards. Some other reasons may be the large numbers of habitat, and less speed. So, the mortality rate is going on the large

numbers. Some reptiles such as snake come to roads for thermoregulation that leads to their death by vehicles (Akrim et al., 2019).

Many tools can be used to control the road mortality of common wild life. Some steps that can be taken immediately in large populated roads of Pakistan are fencing around the road as done on M1 and M2 motorway. It may be expensive but it could be less expensive when local texture used. Speed limit boards are required on each road and fine should be imposed on over speeding by the vehicles. Speed boards have been used by the highway authority or 1124 station on Bhalwal road Sargodha. Also removing of tree top can improve the visibility of animals and can help them to avoid road kills. Although more practical solutions are required to reduce the number of killed animals.

CONCLUSION

This study helped us to understand traffic as a major threat to wildlife. As wildlife is very important for the ecosystem. Moreover, management should nee to involve at national level in order to secure these animals. Limited research is carried out so far in Pakistan, so, there is need to develop practical solutions to conserve wildlife of Pakistan.

ETHICAL APPROVAL

The study was approved by the intuitional ethical review committee.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

1. Ahmad S, Hameed S, Ali H, Khan TU, Mehmood T, Nawaz MA (2016). Carnivores diversity and conflicts with humans in musk deer national park, Azad Jammu and Kashmir, Pakistan. Eur. J. Wildl. Res. 62: 565-576.
2. Akrim F, Tariq M, Shaista A, Riaz H, Wendy JC (2019). Spatiotemporal patterns of wildlife road mortality in the Pothwar Plateau, Pakistan. Mammalia. 83: 487-495.
3. Ashley EP, Robinson JT (1996). Road mortality of amphibians,

reptiles and other wildlife on the Long Point Causeway, Lake Erie, Ontario. Can Field Nat. 110: 403-412.

4. Beebee TJC (2013). Effects of road mortality and mitigation measures on amphibian populations. Conserv. Biol. 27:657–668.
5. Bennett VJ (2017). Effects of road density and pattern on the conservation of species and biodiversity. Curr. Landscape. Ecol. Rep. 2: 1-11.
6. Blaustein AR, Wake DB, Sousa WP (1994). Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. Conserv biol. 8: 60-71.
7. Clevenger T, Huijser MP (2009). Handbook for design and evaluation of wildlife crossing structures in North America.
8. Coffin AW (2007). From roadkill to road ecology: A review of the ecological effects of roads, Journal of Trans Geo. 15: 396-406.
9. Eigenbrod F, Hecnar SJ, Fahrig L (2009). Quantifying the road-effect zone: threshold effects of a motorway on anuran populations in Ontario, Canada. Ecol Soc. 14. 1-24.
10. Fahrig L, Rytwinski T (2009). Effects of roads on animal abundance: an empirical review and synthesis. Ecol Soc. 14: 1-21.
11. Forman RT, Deblinger RD (2003). The ecological road-effect zone of a Massachusetts (USA) suburban highway. Conser biol. 14: 36-46.
12. Gregory A, Spence E, Beier P, Garding E (2021). Toward best management practices for ecological corridors. Land. 10: 140.
13. Grosman PD, Jaeger JA, Biron PM, Dussault C, Ouellet JP (2009). Reducing moose–vehicle collisions through salt pool removal and displacement: an

agent-based modeling approach. Ecol Soc. 14: 1-23.

14. Mazerolle MJ (2004). Amphibian road mortality in response to nightly variations in traffic intensity. Herpetologica. 60: 45-53.
15. Oddone AGHE, Nkomo SL (2021). Spatio-temporal patterns and consequences of road kills: a review. Animals.11: 799.
16. Rehman E, Khattak RH (2020). Trophy Hunting Impacts on Kashmir Markhor and Changing the Negative Perception of Local Communities about Wildlife in Chitral District, Pakistan. Mammal Tales #20, Zoo's Print. 35: 12–14.
17. Rytwinski T, Soanes K, Jaeger JAG, Fahrig L, Findlay CS (2016). How effective is road mitigation at reducing road-kill? A Meta-Analysis. Plos One. 11.
18. Seshadri KS, Yadav A, Gururaja KV (2009). Road kills of amphibians in different land use areas from Sharavathi river basin, central Western Ghats, India. J. Threat. Taxa. 1: 549-552.
19. Younus S, Nazer S, Altaf M, Manzoor I, Safeer B, Yasrub S (2018). Study of human and asiatic jackal (*Canis aureus*) conflict from Bagh District, Azad Jammu and Kashmir, Pakistan. J. Wildl. Ecol. 2: 1–10.

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Association of Obesity with the Occurrence of Gastrointestinal Cancer- A Meta-Analysis

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ABSTRACT: *Obesity is a risk factor for many diseases especially cancer. Numerous studies have been performed to examine the relation between obesity and different types of gastrointestinal cancer. However, involvement of obesity in overall gastrointestinal cancer risk is not very clear. Therefore, a meta-analysis was performed to investigate the association of obesity and overall gastrointestinal cancer risk. A thorough systematic search were performed on PubMed, MEDLINE and other databases and relevant studies were identified and scrutinised. A random effect model was used to calculate the correlation using risk ratio (RR) at 95% confidence interval. Publication bias was assessed by funnel plots. A total of 56 studies were used to perform meta-analysis. The pooled risk ratio calculated showed a significant relation between obesity and gastrointestinal cancer risk (RR = 1.742, 95% CI =1.54 - 1.96, P< 0.001). Subgroup analysis was also performed for different types of gastrointestinal cancer such as oesophageal cancer, stomach cancer, liver cancer, pancreatic cancer, and colorectal cancer. The pooled risk ratio for each type of gastrointestinal cancer was found to be RR = 2.376 (oesophageal cancer), RR = 1.131 (stomach cancer), RR = 1.976 (liver cancer), RR = 1.474 (pancreatic cancer), and RR = 1.428 (colorectal cancer). There was observed no significant bias in the study. This study suggested that obesity is significantly associated with risk of gastrointestinal cancer especially oesophageal cancer. However, further investigations and large clinical trials are required to make an impactful and conclusive statement about this association.*

Keywords: *Obesity; risk ratio; gastrointestinal cancers; Oesophageal cancer; meta-analysis*

INTRODUCTION

Gastrointestinal cancer is the cancer of gastrointestinal track particularly of oesophagus, stomach, small and large intestine, colon, rectum and related organs as liver, pancreas, and gallbladder (Klint et al., 2010, Siegel RL et al., 2017). This cancer like other cancer types shows poor prognosis and is often detected when it has reached to the advanced stages (Arnold et al., 2020).

The prevalence of gastrointestinal cancer vary among different populations due to significant geographical variations. For example, China alone shares 54% of the global burden of oesophagus squamous cell carcinoma (OSCC), a type of oesophageal cancer. It is found that the gastrointestinal cancer is more prevalent in the developed countries like United States, Japan, China etc (Prasad and Tyagi, 2015). According to statistics, it accounts for 18.7% of global cancer incidence and mortality rates of 22.6% in 2020 (YumoXie et al., 2021) and is more common among men as compared to women. The common risk factors of gastrointestinal cancer includes infections, smoking, fatty diet, alcohol consumption, age, gender, race, family history, and the area of prevalence (Arnold et al., 2020; Ilic and Ilic, 2002).

According to WHO, obesity is defined as the excessive accumulation of fat in the body which necessarily can affect the normal health status and is determined by body mass index (BMI). Obesity is common due to the modernization and economic stability in the world which ensures the availability of affordable and excess food to everyone and physical inactivity (Ng et al., 2012). Furthermore, it is not age restricted and is present everywhere regardless of geography, ethnicity, and economic status (Chooi et al., 2019). Obesity presents different physiological problems and contribute to the development of several diseases particularly different types of gastrointestinal cancer (Blüher, 2019; Krupa-Kotara and Dakowska, 2021). World Cancer Research Fund (WCRF) and American Institute for Cancer Research (AICR) in 2018 issued a Continuous Update Project (CUP), a joint review indicating that there is mounting evidence that creates a nexus between obesity and esophagus adenocarcinoma, pancreatic, liver, colorectal and kidney cancer.

Various studies have been performed to observe the relation between obesity and different types of

gastrointestinal cancer, but few showed a pooled effect. These studies showed diverse results making it difficult to present a statement about role of obesity in gastrointestinal cancer risk. Therefore, this meta-analysis was performed to elucidate the association between obesity and occurrence of gastrointestinal cancer.

METHODS

a- Literature Search Strategy

Various search engines like PubMed, MEDLINE and others were searched for studies showing the association between obesity and gastrointestinal cancer. Studies were also included from the citations in the selected papers and review articles. The key words for these searches included 'obesity and risk of Gastrointestinal cancer', 'BMI and risk of Gastrointestinal cancer', 'overweight and risk of Gastrointestinal cancer', 'obesity and risk of esophageal cancer', 'obesity and risk of gastric cancer', 'obesity and risk of stomach cancer', 'obesity and risk of pancreatic cancer', 'obesity and risk of liver cancer', 'obesity and risk of colorectal cancer', 'obesity and risk of gallbladder cancer'.

b- Selection Criteria:

Those published studies were included in our meta-analysis which met the following criteria: (1) studies had to be cohort or case–control study in which gastrointestinal cancer (esophageal, stomach, liver, pancreatic, colorectal) incidence or mortality was taken as outcome; (2) the exposure included overweight and/or obesity defined by body mass index (BMI) (the weight in kilograms divided by the square of height in meters), (3) estimates of relative risk (hazard ratio, odds ratio) and relative risk (RR) for at least 3 categories of BMI were reported in studies.

c- Literature Retrieval and Data Extraction:

Initially, 135 studies were chosen through search of different databases. Out of these, 73 studies were excluded from further evaluation because their abstract did not contain the required information for our data. Remaining 62 studies were evaluated for full text assessment. Out of these further 6 studies were excluded due to missing data. Finally, 56 studies were found eligible for performing meta-analysis and required data was extracted from these studies.

From each study, the following information was extracted: Author's name, publication year, country where study was performed; study design (cohort or case–control), sample size,

age range of participants, number of patients, categories of body mass index, relative risk for each category of body mass index, gender, type of Gastrointestinal cancer, outcome as yes or no, estimated relative risk with 95% Cl. The adjusted relative risks were noted where available.

d- Exposure definition

BMI ranges as specified by the WHO was used i.e. 18.5 to 24.9kg/m^2 range represents the normal BMI, whereas BMI greater than 25 kg/m 2 represents overweight persons and a BMI greater than 30 kg/m^2 represent obese. If BMI exceeds 40 kg/m^2,it is classified as severe obesity (Chooi et al., 2019).

e- Statistical Analysis

Random effect model was used to calculate the summary or pooled risk ratio (RR) estimate with 95% Cls. Some studies represented results in the form of hazard ratio (HR) and odd ratio (OR), but these association measures were considered as risk ratio in our study. There were studies like Calle et al, 2003, and others which published RR for obesity group (>30 kg/m) in more than one category such as a risk ratio estimate for 30 to 35 kg/m^2 in one category, another risk estimate for 35 to 40 kg/m^2 categorised as obese II or severe obese and so on. For these a mean of all the relative risk estimates was taken and used in the meta-analysis. Also, some papers published RR separately for male and female, for these again a mean risk ratio was taken. Most adjusted risk ratio value was taken. The assessment of heterogeneity in the selected studies was done by using Q and I^2 statistics. Publication bias was assessed using funnel plot. All analyses were performed using the Comprehensive Meta Analysis (CMA) Version 3.0.

RESULTS

a- Literature retrieval and Characteristics of included Studies:

After thoroughly analyzing 135 studies finally 56 studies were selected for performing meta-analysis (Figure 1). The studies included in the meta-analysis were published between 1995 and 2018 and contain both cohort and case control studies. It was observed that these studies were performed in almost every region of the world including US, England, Netherlands, Australia, Sweden, Israel, Singapore, Norway, Japan, China, Australia, Korea, Italy, Canada, Ireland, and South Korea and all studies included in the meta-analysis had been seen to report result on only one of different types of

gastrointestinal cancer (oesophagus cancer, gastric cancer, liver cancer, pancreatic cancer, and colorectal cancer) but some studies like (Pan et al., 2003; Jee et al., 2008; Batty et al., 2005) reported for every type of gastrointestinal cancer. So, for these studies we have entered entries for each cancer type separately. Also, most studies used Cox regression model and Cox proportional hazard model to find relative risk (RR) between obesity and different type of gastrointestinal cancer. Other main characteristics of the selected studies were shown in table 1.

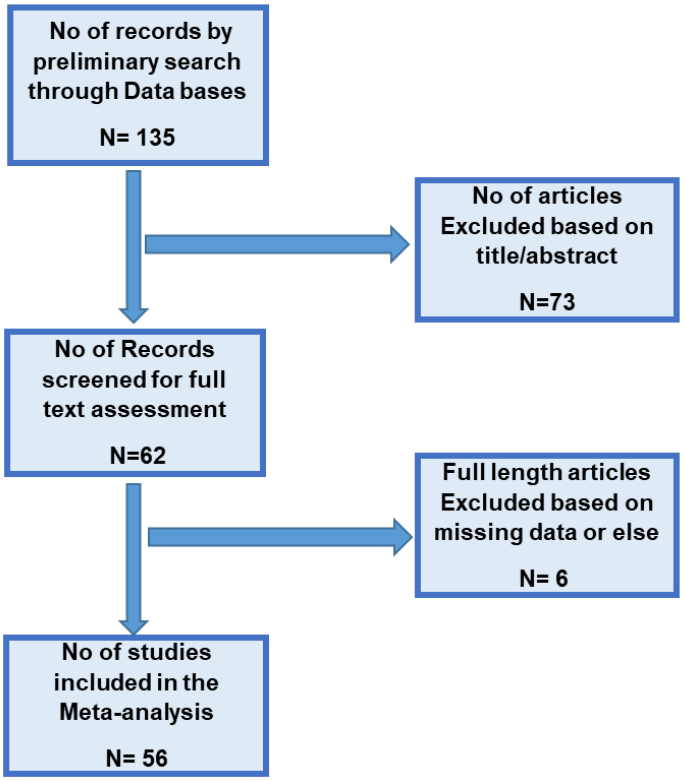


Fig. 1: Flowchart representing the steps of the literature search

Table 1: Characteristics of the Eligible studies

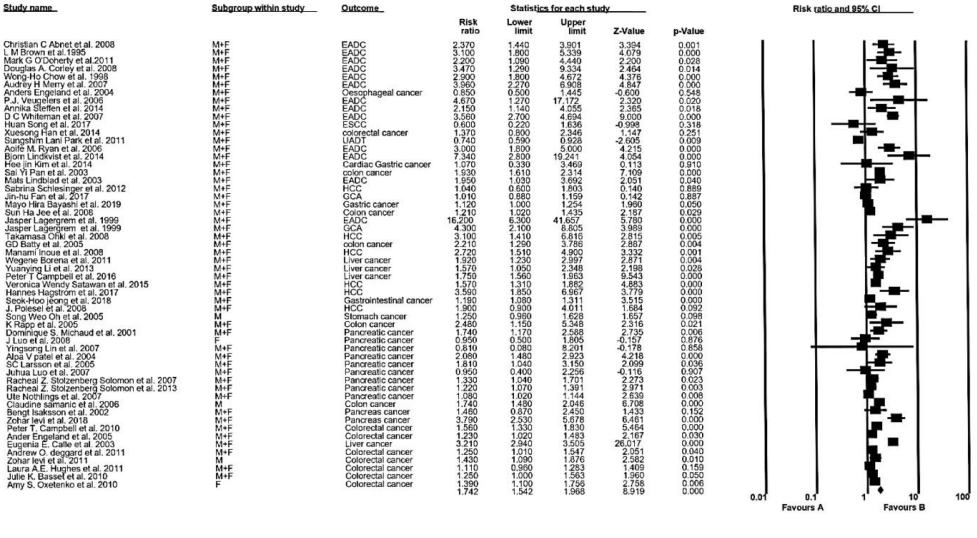
	Study		No. of participants		Type of study	No. of patients with BMI				Gender	Type of GIT cancer	Age	Geo Location	RR
	Author	Year	Control	Patients		Under	Normal	Over	Obese					
1	Abnet et al.	2008	480,475	371	Cohort	2 (1.61)	71 (1.00)	194 (1.65)	104 (2.09)	M+F	EADC	50-71	USA	2.37 (1.44–3.59)
2	Brown et al.	1995	750	174	Case control	24 (1.00)	31 (1.1)	27 (1.2)	79 (3.1)	M	EADC	30-79	USA	3.1 (1.8-5.3)
3	O'Doherty et al.	2011	218854	253	Cohort	0	59 (1.00)	119 (1.3)	75 (2.20)	M+F	EADC	50-71	USA	2.20 (1.09 to 4.09)
4	Corley et al.	2008	206974	101	Nested Case-control	8 (1.00)	25 (2.09)	22 (3.47)	?	M+F	EADC	?	USA	3.47 (1.29-9.33)
5	Chow et al.	1998	695	554	Case-control	45 (1.00)	63 (1.3)	85 (2.00)	99 (2.90)	M+F	EADC	30-79	USA	2.90 (1.8–4.7)
6	Merry et al.	2007	120852	133	Cohort	3 (1.29)	51 (1)	60 (1.40)	19 (3.96)	M+F	EADC	55-69	Nether land	3.96 (2.27 to 6.88)
7	Engeland et al.	2004	2 million	2245	Cohort	40 (2.46)	1208 (1.00)	827 (0.82)	170 (0.85)	M+F	Oesophag-eal cancer	20-74	Norway	0.85 (0.50–0.82)
8	Veugelers et al.	2006	102	57	Case-control	0	16% (1.00)	43% (1.58)	41% (4.67)	M+F	EADC	?	Canada	4.67 (1.27–17.19)
9	Steffen et al.	2014	395456	124	Cohort	37 (1.15)	24 (1.36)	30 (1.76)	33 (2.15)	M+F	EADC	20-70	UK	2.15 (1.14–4.05)
10	Whiteman et al.	2007	1580	801	Case-control	1 (0.5)	71 (1.00)	150 (1.2)	130 (3.56)	M+F	EADC	18-79	Australia	3.56 (2.7 to 13.6)
11	Song et al.	2017	96331	342	cohort	12 (0.87)	274 (1.00)	52 (0.57)	4 (0.60)	M+F	ESCC	40-69	Japan	0.60 (0.22–1.61)
12	Han et al.	2014	13901	298	Cohort	13 (0.87)	210 (1.00)	58 (1.07)	17 (1.37)	M	Colorectal	45-64	USA	1.37 (0.8-3.11)
13	Park et al.	2011	2173	2048	Case-control	57 (2.10)	785 (1.00)	613 (0.74)	252 (0.74)	M+F	UADT	?	Europe	0.74 (0.59 - 0.93)
14	Ryan et al.	2006	893	283	Case-control	40 (1.00)	43 (1.00)	74 (1.9)	131 (3.00)	M+F	EADC	?	Ireland	3.0 (1.8–5.0)
15	Lindkvist et al.	2014	578700	114	Cohort	5 (1.00)	36 (3.27)	31 (5.19)	42 (7.34)	M+F	EADC	?	Australia Norway Sweden	7.34 (2.8–18.68)
16	Kim et al.	2014	1288	998	Case-control	468 (1.00)	266 (1.08)	244 (1.22)	26 (1.07)	M+F	Cardiac Gastric	30-80	South Korea	1.07 (0.331–2.255)
17	Pan et al.	2003	5039	1176	Case-control	?	?	1176 (0.97)	1176 (1.25)	M+F	Stomach	20-76	Canada	1.25 (1.03, 1.51)
				1722		?	?	1722 (1.4)	1722 (1.93)		Colon	20-76		1.93 (1.61, 2.31)
				1447		?	?	1447 (1.36)	1447 1.65		Rectum	20-76		1.65 (1.36, 2.00)
				630		?	?	630 0.99	630 (1.51)		Pancreatic	20-76		1.51 (1.19, 1.92)
				309		?	?	309 (0.89)	309 (1.17)		Liver	20-76		1.17 (0.83, 1.66)
18	Lindblad et al.	2003	10,000	287	Case-control	8 (1.29)	49 (1.00)	94 (1.47)	36 (1.95)	M+F	EADC	40-84	Sweden	1.95 (1.03–3.02)
19	Schlesinger et al.	2012	359525	177	Cohort	?	33 (1.00)	49 (0.96)	95 (1.04)	M+F	HCC	?	Europe	1.04 (0.60–1.83)
20	Fan et al.	2017	29446	1716	Cohort	403 (1.00)	442 (1.02)	390 (1.01)	?	M+F	GCA	?	China	1.01 (0.88–1.16)
21	Bayashi et al.	2019	92056	2860	Cohort	145 (1.17)	1988 (1.00)	426 (1.00)	301 (1.12)	M+F	Gastric cancer	40-69	Japan	1.12 (1.00 - 1.51)
22	Jee et al.	2008	1213829	13810	Cohort	2332 (0.95)	11790 (1.01)	4320 (0.98)	242 (1.07)	M+F	Stomach	30-95	Korea	1.07 (1.05–1.64)
				1231		291 (2.11)	1033 (1.12)	265 (1.52)	5 (1.48)		Oesophagus	30-95		1.48 (0.51–11.7)
				4706		612 (0.72)	4100 (0.99)	1791 (1.00)	127 (1.21)		Colon	30-95		1.21 (1.02–1.98)
				7646		1057 (0.87)	6639 (0.92)	2649 (1.09)	175 (1.51)		Liver	30-95		1.51 (1.27–2.10)
				1959		279 (0.87)	1626 (1.02)	705 (1.20)	51 (1.57)		Pancreatic	30-95		1.57 (0.75–2.38)
				2406		367	2021	883	67		Gallbladder	30-95		1.54

						(0.88)	(0.99)	(1.12)	(1.54)					(1.11–2.44)
23	Lagergrem etal.	1999	820	189	Case-control	10 (1.00)	68 (3.20)	89 (6.90)	22 (16.20)	M+F	EADC	<80	Sweden	16.20 (6.3–41.4)
24	Lagergrem et al.	1999	820	262	Case-control	47 (1.00)	100 (1.30)	91 (2.20)	34 (4.30)	M+F	GCA	<80	Sweden	4.30 (2.1–8.7)
25	Ohki et al.	2008	1431	340	Cohort	112 (1.00)	1023 (1.52)	265 (1.86)	31 (3.10)	M+F	HCC	?	Japan	3.10 (1.41–6.81)
26	Batty et al.	2005	18403	279	Cohort	?	139 (1.00)	122 (1.20)	18 (2.21)	M+F	Colon	?	UK	2.21 (1.29, 3.79)
				190		?	100 (1.00)	81 (1.05)	9 (1.23)		Stomach	?		1.23 (0.59, 2.58)
				147		?	75 (1.00)	69 (1.18)	3 (0.58)		Pancreatic	?		0.58 (0.18, 1.91)
27	Inoue et al.	2008	17590	102	Cohort	?	64 (1.00)	21 (2.07)	17 (2.72)	M+F	HCC	40-69	Japan	2.72 (1.51–4.89)
28	Borena et al.	2011	578200	266	Cohort	36 (1.00)	83 (0.94)	53 (1.02)	94 (1.92)	M+F	liver	?	Norway Austria	1.92 (1.23–2.96)
29	Li et al.	2013	72468	527	Cohort	23 (1.63)	166 (1.36)	65 (1.57)	?	M+F	liver	40-79	Japan	1.57 (1.05–2.60)
30	Campbell et al.	2016	1.57 million	2162	Prospe-ctive	19 (1.41)	586 (1.00)	861 (1.17)	621 (1.75)	M+F	liver	58.2	USA	1.75 (1.56–1.98)
31	Satawan et al.	2015	168476	482	Cohort	?	152 (1.00)	216 (1.24)	114 (1.57)	M+F	HCC	45-77	USA	1.57 (1.31–2.52)
32	Hagstrom et al.	2017	1.2 million	251	Cohort	31 (1.12)	185 (1.14)	25 (1.57)	10 (3.59)	M+F	HCC	?	Sweden	3.59 (1.85 to 6.99)
33	Jeong et al.	2018	510148	7831	Cohort	?	5350 (0.81)	?	2481 (1.19)	M+F	Gastro-intestinal	45-77	Korea	1.19 (1.08, 1.32)
34	Polesel et al.	2008	404	185	Case-control	?	71 (1.00)	76 (1.00)	38 (1.90)	M+F	HCC	40-82	Italy	1.90 (0.9–3.9)
35	Oh et al.	2005	781283	187	Cohort	106 (0.96)	3871 (0.92)	1260 (0.94)	56 (1.25)	M	Stomach	?	Korea	1.25 0.96 to 1.63
36	Rapp et al.	2005	145000	146	Cohort	?	58 (1.00)	75 (1.04)	13 (0.72)	M+F	Stomach	?	Austria	0.72 (0.40–1.33)
				221		?	86 (1.00)	128 (1.56)	7 (2.48)		Colon	?		2.48 (1.15–5.39)
				57		?	18 (1.00)	29 (1.32)	10 (1.67)		Liver	?		1.67 (0.75–3.72)
				64		?	19 (1.00)	31 (1.29)	14 (2.34)		Pancreas	?		2.34 (1.17–4.66)
37	Michaud et al.	2001	163691	350	Cohort	104 (1.00)	73 (1.08)	126 (1.36)	47 (1.74)	M+F	Pancreas	30-75	USA	1.74 (1.17–4.66)
38	Luo et al.	2008	138503	251	Cohort	25 (0.8)	62 (1.00)	84 (0.90)	80 (0.95)	F	Pancreas	50-79	USA	0.95 (0.5 – 1.3)
39	Lin et al.	2007	110792	402	Cohort	79 (1.13)	250 (1.06)	78 (1.09)	5 (0.81)	M+F	Pancreas	?	Japan	0.81 (0.08–4.16)
40	Patel et al.	2004	145627	242	Cohort	?	94 (1.00)	90 (1.03)	58 (2.08)	M+F	Pancreas	?	USA	2.08 (1.48-2.93)
41	Larsson et al.	2005	83140	128	Cohort	5 (0.96)	50 (1.00)	54 (1.25)	19 (1.81)	M+F	Pancreas	?	Sweden	1.81 (1.04 – 3.15)
42	Luo et al.	2007	99670	224	Cohort	51 (1.15)	118 (1.00)	55 (0.95)	?	M+F	Pancreas	?	Japan	0.95 (0.4–1.2)
43	Solomon et al.	2007	495035	654	Cohort	?	194 (1.00)	311 (1.26)	149 (1.33)	M+F	Pancreas	50-71	USA	1.45 (1.04, 2.02)
44	Solomon et al.	2013	501698	2122	Cohort	25 (1.18)	689 (1.00)	934 (1.09)	474 (1.22)	M+F	Pancreas	50-71	USA	1.22 (1.07, 1.55)
45	Nothlings et al.	2007	167430	472	Cohort	?	245 (1.00)	156 (0.89)	75 (1.08)	M+F	Pancreas	?	USA	1.08 (1.02, 2.26)
46	Samanic et al.	2006	362552	320	Cohort	?	184 (1.00)	110 (0.76)	26 (1.14)	M	Oesophagus	34.3	Sweden	1.14 (0.76–1.73)
				1362		?	626 (1.00)	610 (1.08)	126 (1.36)		Rectum	34.3		1.36 (1.13–1.66)
				297		?	115 (1.00)	126 (1.29)	56 (3.62)		Liver	34.3		3.62 (2.62–5.00)
				698		?	352 (1.00)	289 (0.95)	57 (1.16)		Pancreas	34.3		1.16 (0.87–1.53)
				1795		?	763 (1.00)	842 (1.24)	190 (1.74)		Colon	34.3		1.74 (1.48–2.04)
47	Isaksson et al.	2002	21884	176	Cohort	25 (1.02)	31 (1.00)	35 (1.28)	36 (1.46)	M+F	Pancreas	56	Sweden	1.46 (0.87–2.45)
48	Zohar Levi	2018	1794570	551	Cohort	39	420	465	36	M+F	Pancreas	?	Israel	3.79

	et al.					(1.33)	(1.00)	(1.53)	(3.79)					(2.53-5.36)
49	Campbell et al.	2010	2684	1794	Case-control	26 (1.14)	627 (1.00)	660 (1.16)	434 (1.56)	M+F	Colorectal	?	USA	1.56 (1.33 to 2.40)
50	Engeland et al.	2005	2 million	47117	Cohort	388 (0.94)	22568 (1.00)	18733 (1.08)	5428 (1.23)	M+F	Colorectal	?	Norway	1.23 (1.32–1.48)
51	Calle et al.	2003	900000	596	Cohort	?	222 (1.00)	296 (1.13)	102 (3.21)	M+F	Liver	39-79	USA	3.21 (2.94–6.94)
52	Deggard et al.	2011	51251	980	Cohort	76 (1.03)	591 (1.06)	199 (1.05)	114 (1.25)	M+F	Colorectal	45-74	Singapore	1.25 (1.01-1.55)
53	Levi et al.	2011	1.1 million	537	Cohort	89 (1.00)	323 (1.01)	125 (1.43)	?	M	Colorectal	?	Israel	1.43 (1.09–1.89)
54	Hughes et al.	2011	120852	2316	Cohort	?	1372 (0.96)	469 (0.98)	476 (1.11)	M+F	Colorectal	55-69	Nether Land	1.11 0.96- 1.62
55	Basset et al.	2010	39548	569	Cohort	77 (0.77)	97 (1.00)	262 (1.07)	133 (1.25)	M+F	Colorectal	40-69	Australia	1.25 (1.00-2.28)
56	Oxetenko et al.	2010	36941	1464	Cohort	19 (1.62)	495 (1.00)	548 (1.12)	402 (1.39)	F	Colorectal	50-69	USA	1.39 (1.10–2.22)

Most of the studies showed a close association between obesity and different types of gastrointestinal cancer. The pooled RR for overall 56 studies in favour of gastrointestinal cancer (GI) risk was recorded as RR = 1.742, 95% (the black diamond) CI (1.54 - 1.96). This shows that obesity is significantly associated with gastrointestinal cancer risk (figure 2a). For the measurement of heterogeneity, the value of I was I^2 = 92.8% 92% of observed variance between studies is due to real difference in effect size and only 08% of observed variance should be expected to base on random error and tau^2 value was 0.157.No obvious asymmetry was found in the funnel plot as evident from the figure 2b indicating no significant biasness in the studies included in meta-analysis.

(a)



(b)

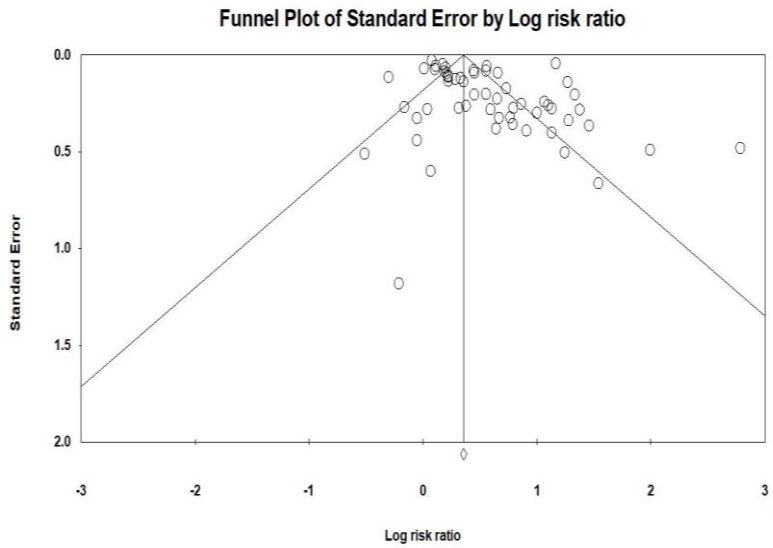


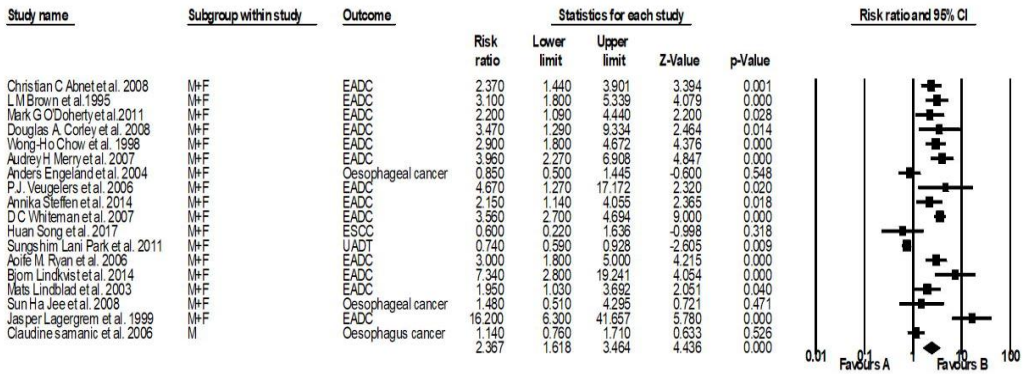
Fig. 2: (a)Forest plot of Risk ratio with a random-effects model for pooled risk ratios of gastrointestinal cancers (Favours A= little or no risk, Favours B= gastrointestinal cancer risk) (b) Funnel plot of risk ratio with a random-effects model for overall biasness in included studies

Following are the results for subgroup analysis performed separately for each gastrointestinal cancer type.

b- Oesophagus Cancer

The pooled RR for oesophageal cancer risk was found to be, RR = 2.376, 95% CI (1.61 – 3.46) showing a significant association between obesity and oesophagus cancer. The I^2 = 89.9% showing heterogeneity among studies and tau^2 = 0.55 as variance measure. Figure 3a,b shows the forest plot and funnel plot for association between obesity and oesophagus cancer and publication bias which is insignificant.

(a)



(b)

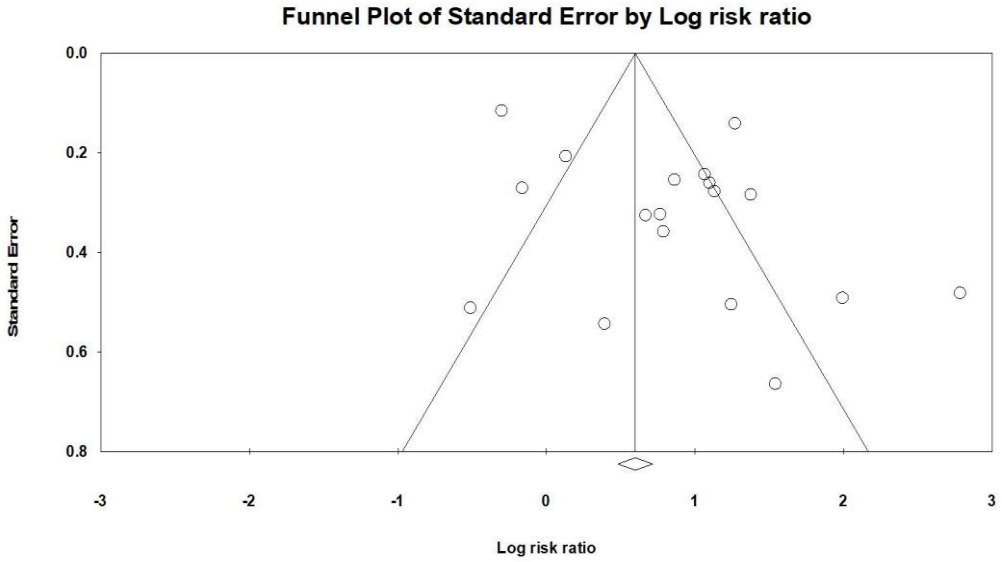
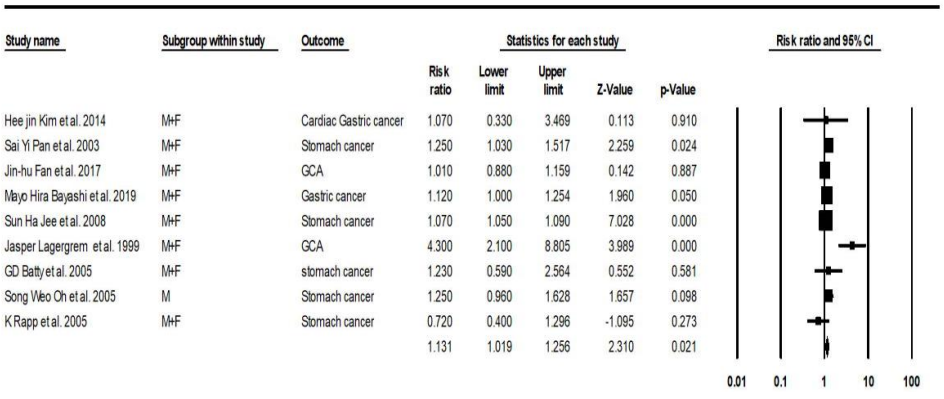


Fig. 3: (a) Forest plot of risk ratio with a random-effects model for risk ratio in oesophagus cancer studies (b) Funnel plot of risk ratio with a random-effects model for overall biasness in oesophagus cancer studies

c- Stomach/ Gastric cancer

The pooled RR for stomach cancer risk was RR = 1.131, 95% CI (1.01 – 1.25) showing a positive association between obesity and stomach cancer. The I^2 = 62.5% showing heterogeneity among studies and tau^2 = 0.01 as variance measure. Figure 4a, b shows the forest plot and funnel plot for association between obesity and stomach cancer and publication bias which is insignificant.

(a)



(b)

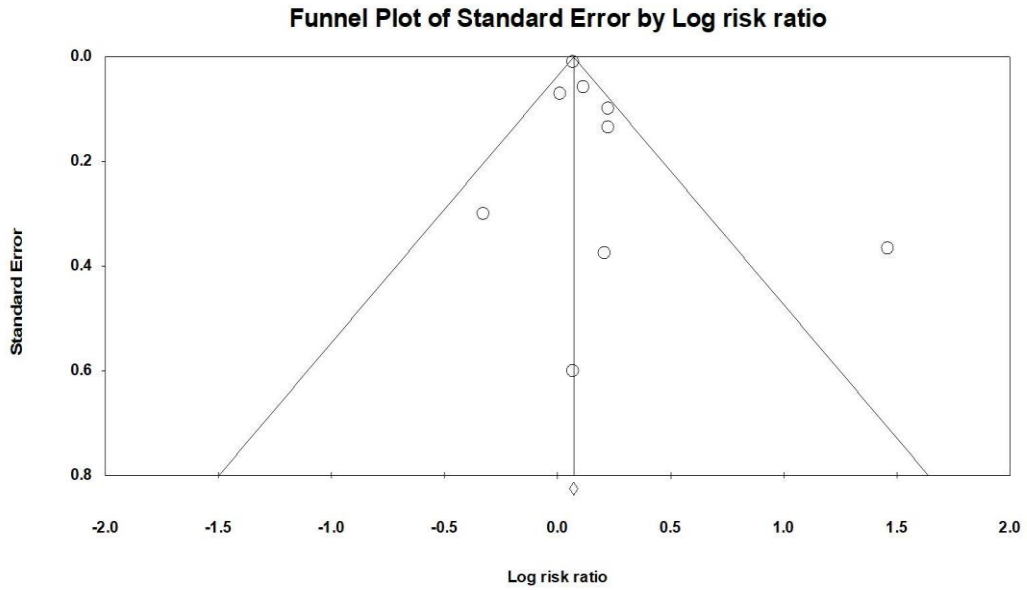
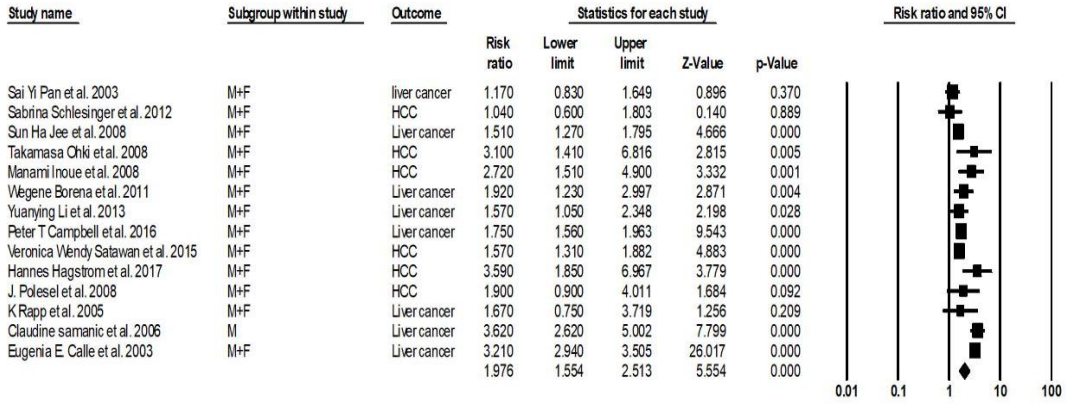


Fig. 4: (a) Forest plot of risk ratio with a random-effects model for risk ratio in stomach cancer studies (b) Funnel plot of risk ratio with a random-effects model for overall biasness in stomach cancer studies

d- Liver Cancer

The pooled RR for liver cancer risk was RR = 1.976, 95% CI (1.55 – 2.51) showing a significant association between obesity and liver cancer. The I^2 = 91.5% showing heterogeneity among studies and tau^2 = 0.15 as variance measure. Figure 5a, b shows the forest plot and funnel plot for association between obesity and liver cancer and publication bias which is insignificant.

(a)



(b)

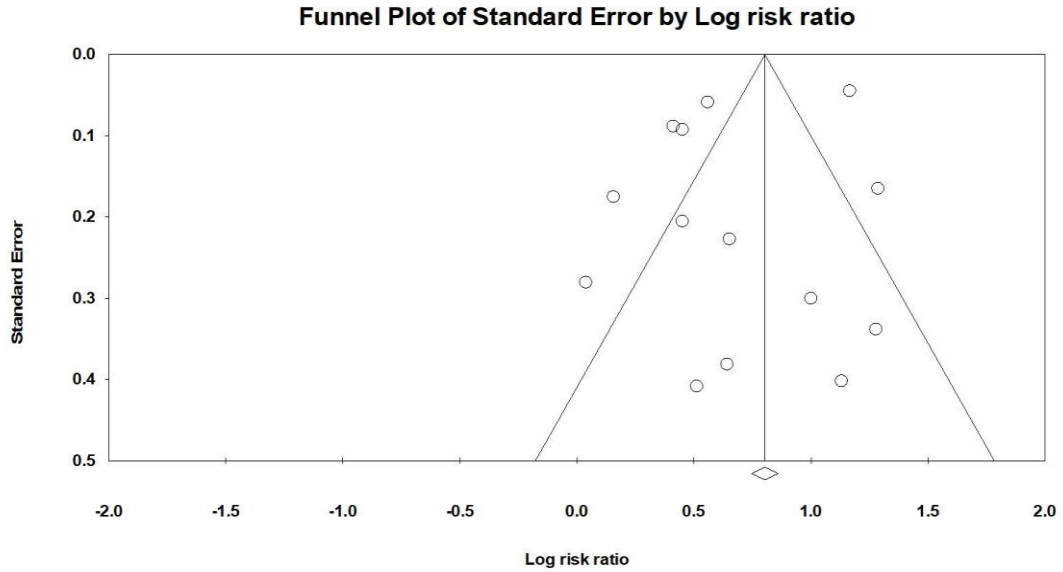
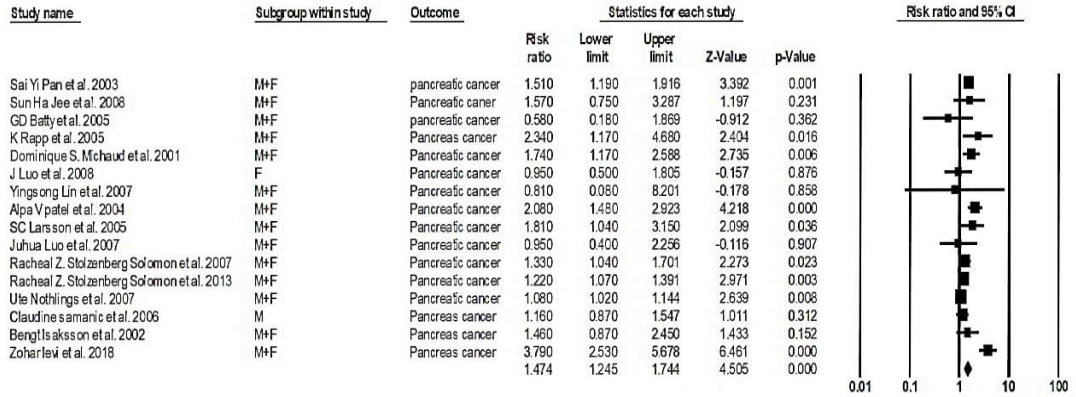


Fig. 5: (a) Forest plot of risk ratio with a random-effects model for risk ratio in liver cancer studies (b) Funnel plot of risk ratio with a random-effects model for overall biasness in liver cancer studies

e- Pancreatic Cancer

The pooled RR for pancreatic cancer risk was RR = 1.474, 95% CI (1.24 – 1.74) showing a significant association between obesity and pancreatic cancer. The I^2 = 78% showing heterogeneity among studies and tau^2 = 0.06 as variance measure. Figure 6a, b shows the forest plot and funnel plot for association between obesity and pancreatic cancer and publication bias which is insignificant.

(a)



Study name	Subgroup within study	Outcome	Statistics for each study: Risk ratio	Lower limit	Upper limit	Z-Value	p-Value
Sai Yi Pan et al. 2003	M+F	pancreatic cancer	1.510	1.190	1.916	3.392	0.001
Sun Ha Jee et al. 2008	M+F	Pancreatic caner	1.570	0.750	3.287	1.197	0.231
GD Batty et al. 2005	M+F	pancreatic cancer	0.580	0.180	1.869	-0.912	0.362
K Rapp et al. 2005	M+F	Pancreas cancer	2.340	1.170	4.680	2.404	0.016
Dominique S. Michaud et al. 2001	M+F	Pancreatic cancer	1.740	1.170	2.588	2.735	0.006
J Luo et al. 2008	F	Pancreatic cancer	0.950	0.500	1.805	-0.157	0.876
Yingsong Lin et al. 2007	M+F	Pancreatic cancer	0.810	0.080	8.201	-0.178	0.858
Alpa V patel et al. 2004	M+F	Pancreatic cancer	2.080	1.480	2.923	4.218	0.000
SC Larsson et al. 2005	M+F	Pancreatic cancer	1.810	1.040	3.150	2.099	0.036
Juhua Luo et al. 2007	M+F	Pancreatic cancer	0.950	0.400	2.256	-0.116	0.907
Racheal Z. Stolzenberg Solomon et al. 2007	M+F	Pancreatic cancer	1.330	1.040	1.701	2.273	0.023
Racheal Z. Stolzenberg Solomon et al. 2013	M+F	Pancreatic cancer	1.220	1.070	1.391	2.971	0.003
Ute Nothlings et al. 2007	M+F	Pancreatic cancer	1.080	1.020	1.144	2.639	0.008
Claudine samanic et al. 2006	M	Pancreas cancer	1.160	0.870	1.547	1.011	0.312
Bengt Isaksson et al. 2002	M+F	Pancreas cancer	1.460	0.870	2.450	1.433	0.152
Zohar levi et al. 2018	M+F	Pancreas cancer	3.790	2.530	5.678	6.461	0.000
			1.474	1.245	1.744	4.505	0.000

(b)

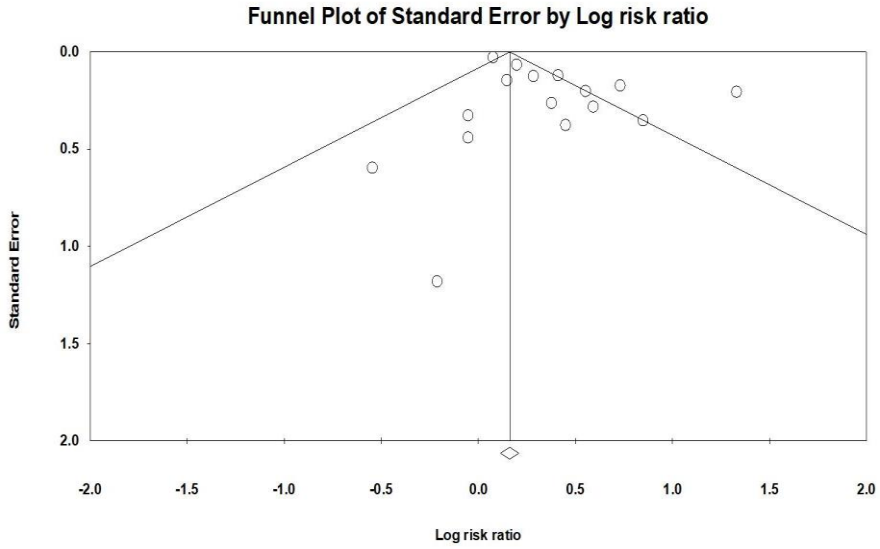


Fig. 6: (a) Forest plot of risk ratio with a random-effects model for risk ratio in pancreatic cancer studies (b) Funnel plot of risk ratio with a random-effects model for overall biasness in pancreatic cancer studies

f- Colorectal Cancer

The pooled RR for colorectal cancer risk was RR = 1.428, 95% CI (1.27 – 1.60) showing a significant association between obesity and colorectal cancer. The I^2 = 71% showing heterogeneity among studies and tau^2 = 0.029 as variance measure. Figure 7 shows the forest plot and funnel plot for association between obesity and colorectal cancer and publication bias which is insignificant.

(a)

Study name	Subgroup within study	Outcome	Statistics for each study					Risk ratio and 95% CI
			Risk ratio	Lower limit	Upper limit	Z-Value	p-Value	
Xuesong Han et al. 2014	M+F	colorectal cancer	1.370	0.800	2.346	1.147	0.251	
Sai Yi Pan et al. 2003	M+F	colon cancer	1.930	1.610	2.314	7.109	0.000	
Sun Ha Jee et al. 2008	M+F	Colon cancer	1.210	1.020	1.435	2.187	0.029	
GD Batty et al. 2005	M+F	colon cancer	2.210	1.290	3.786	2.887	0.004	
K Rapp et al. 2005	M+F	Colon cancer	2.480	1.150	5.348	2.316	0.021	
Claudine samanic et al. 2006	M	Colon cancer	1.740	1.480	2.046	6.708	0.000	
Peter T. Campbell et al. 2010	M+F	Colorectal cancer	1.560	1.330	1.830	5.464	0.000	
Ander Engeland et al. 2005	M+F	Colorectal cancer	1.230	1.020	1.483	2.167	0.030	
Andrew O. deggard et al. 2011	M+F	Colorectal cancer	1.250	1.010	1.547	2.051	0.040	
Zohar levi et al. 2011	M	Colorectal cancer	1.430	1.090	1.876	2.582	0.010	
Laura AE. Hughes et al. 2011	M+F	Colorectal cancer	1.110	0.960	1.283	1.409	0.159	
Julie K. Basset et al. 2010	M+F	Colorectal cancer	1.250	1.000	1.563	1.960	0.050	
Amy S. Oxetenko et al. 2010	F	Colorectal cancer	1.390	1.100	1.756	2.758	0.006	
			1.428	1.271	1.606	5.969	0.000	

(b)

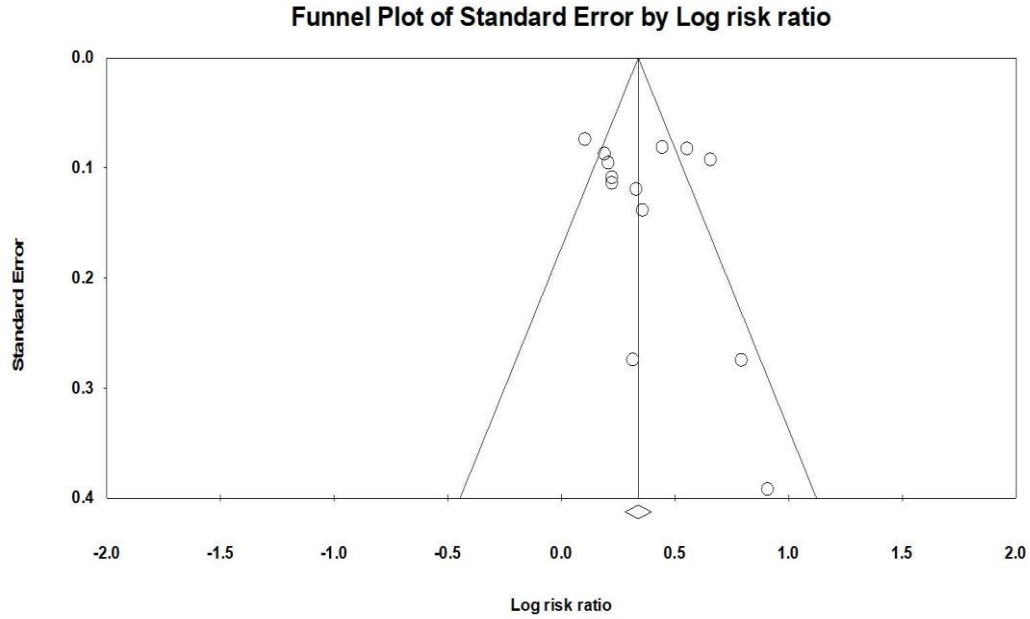


Fig. 7: (a) Forest plot of risk ratio with a random-effects model for risk ratio in colorectal cancer studies (b) Funnel plot of risk ratio with a random-effects model for overall biasness in colorectal cancer studies

DISCUSSION

Gastrointestinal cancer is one of the leading causes of mortality worldwide. According to statistics, gastrointestinal cancer is the reason of death of 1/3 cancer patients (Arnold et al., 2020).Obesity plays an important role in the development of gastrointestinal but a clear relation between two is missing. We performed this meta-analysis with 56 eligible studies after considerable scrutiny to find the association between obesity and risk of gastrointestinal cancer incidence. In almost all studies, BMI was used as a measure of obesity. Most of the studies showed up strong positive association between obesity and different types of gastrointestinal cancer risk and mortality.

The meta-analysis results described that obesity significantly increases the risk of overall gastrointestinal cancer (RR = 1.742). According to results the type of gastrointestinal cancer which was strongly related with obesity was oesophageal cancer compared to all other gastrointestinal cancers (RR = 2.376). Random effect model was applied to determine effect size and heterogeneity. Random effect model considered variations among all studies included in the meta-analysis. Every

study reported a different effect size (RR) due to changes in subject's characteristics of each study.

Weight loss activities for obese people can decrease the overall risk of gastrointestinal cancer. This association between obesity and overall risk of gastrointestinal tract cancers may be due to some mechanisms which are related with adipose tissue production of adipokinesis and vascular growth factors, changes in immune system functions and endocrine disruptors (De Pergola Silvestris, 2013).

Excess adiposity is recognized as the second major cause of cancer, after smoking. There are different hypotheses at present which provide a convincing link between obesity and gastrointestinal cancer. First one is altered insulin signaling. According to this, Excess adiposity leads to insulin resistance causing hyper-insulinemia. High concentration of insulin in blood which is a mitogenic hormone activates MAP-Kinase resulting in cell proliferation causing cancer. Second is chronic inflammation which is the result of excessive accumulation of visceral fat causing improper activation of pro-inflammatory signals and cytokine production. This condition results in the release of free fatty acids in circulation and macrophages. Free fatty acids cause the activation of NF-kB factors which may results in the development of gastric cancer. Adipose tissues stimulate the production of sex hormones particularly estrone and estradiol. It causes the stimulation of IGF-1 receptor and help in cell proliferation causing cancer (Karczewski et al., 2019).

But more deep study is required to fully understand the underlying mechanism (De Pergola Silvestris, 2013). Also, people with BMI less than 25 kg/m^2 were seen to be at lower risk of developing gastrointestinal cancer.

CONCLUSION

It was concluded by this study that obese people were at greater risk of developing different types of gastrointestinal cancer as compared to non-obese even at early age.

ETHICAL APPROVAL

The ethical approval was not implemented.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

1. Abbas G, Krasna M (2017). Overview of esophageal cancer. Ann. Cardiothorac. Surg. 6(2): 131-136. doi: 10.21037/acs.2017.03.03.

2. Abnet CC, Arnold M, Wei WQ (2018). Epidemiology of Esophageal Squamous Cell Carcinoma. Gastroenterology. 154(2): 360-373. doi: 10.1053/j.gastro.2017.08.023.
3. Abnet CC, Freedman ND, Hollenbeck AR, Fraumeni JF Jr, Leitzmann M, Schatzkin A (2008). A prospective study of BMI and risk of oesophageal and gastric adenocarcinoma. Eur. J. Cancer 44(3): 465-71. doi: 10.1016/j.ejca.2007.12.009.
4. Ali R, Barnes I, Cairns BJ, Finlayson AE, Bhala N, Mallath M, Beral V (2013). Incidence of gastrointestinal cancers by ethnic group in England, 2001-2007. Gut. 62(12): 1692-703. doi: 10.1136/gutjnl-2012-303000.
5. Arnold M, Soerjomataram I, Ferlay J, Forman D (2015). Global incidence of oesophageal cancer by histological subtype in 2012. Gut. 64(3): 381-7. doi: 10.1136/gutjnl-2014-308124.
6. Arnold M, Abnet CC, Neale RE, Vignat J, Giovannucci EL, McGlynn KA, Bray F (2020). Global Burden of 5 Major Types of Gastrointestinal Cancer. Gastroenterology. 159(1): 335-349. doi: 10.1053/j.gastro.2020.02.068.
7. Arnold M, Rutherford MJ, Bardot A, Ferlay J, Andersson TM, Myklebust TÅ, Tervonen H, Thursfield V, Ransom D, Shack L, Woods RR, Turner D, Leonfellner S, Ryan S, Saint-Jacques N, De P, McClure C, Ramanakumar AV, Stuart-Panko H, Engholm G, Walsh PM, Jackson C, Vernon S, Morgan E, Gavin A, Morrison DS, Huws DW, Porter G, Butler J, Bryant H, Currow DC, Hiom S, Parkin DM, Sasieni P, Lambert PC, Møller B, Soerjomataram I, Bray F (2019). Progress in cancer survival, mortality, and incidence in seven high-income countries 1995-2014 (ICBP SURVMARK-2): a population-based study. Lancet Oncol. 20(11): 1493-1505. doi: 10.1016/S1470-2045(19)30456-5.
8. Bassett JK, Severi G, English DR, Baglietto L, Krishnan K, Hopper JL, Giles GG (2010). Body size, weight change, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev. 19(11): 2978-86. doi:

10.1158/1055-9965.EPI-10-0543.

9. Batty GD, Shipley MJ, Jarrett RJ, Breeze E, Marmot MG, Smith GD (2005). Obesity and overweight in relation to organ-specific cancer mortality in London (UK): findings from the original Whitehall study. Int J Obes. (Lond). 29(10): 1267-74. doi: 10.1038/sj.ijo.0803020.
10. Bishehsari F, Mahdavinia M, Vacca M, Malekzadeh R, Mariani-Costantini R (2014). Epidemiological transition of colorectal cancer in developing countries: environmental factors, molecular pathways, and opportunities for prevention. World J Gastroenterol. 20(20): 6055-72. doi: 10.3748/wjg.v20.i20.6055.
11. Blüher M (2019). Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol. 15(5): 288-298. doi: 10.1038/s41574-019-0176-8.
12. Borena W, Strohmaier S, Lukanova A, Bjørge T, Lindkvist B, Hallmans G, Edlinger M, Stocks T, Nagel G, Manjer J, Engeland A, Selmer R, Häggström C, Tretli S, Concin H, Jonsson H, Stattin P, Ulmer H (2012). Metabolic risk factors and primary liver cancer in a prospective study of 578,700 adults. Int J Cancer. 131(1): 193-200. doi: 10.1002/ijc.26338.
13. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 68(6): 394-424. doi: 10.3322/caac.21492.
14. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ (2003). Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med. 348(17): 1625-38. doi: 10.1056/NEJMoa021423.
15. Campbell PT, Jacobs ET, Ulrich CM, Figueiredo JC, Poynter JN, McLaughlin JR, Haile RW, Jacobs EJ, Newcomb PA, Potter JD, Le Marchand L, Green RC, Parfrey P, Younghusband HB, Cotterchio M, Gallinger S, Jenkins MA, Hopper JL, Baron JA, Thibodeau SN, Lindor NM, Limburg PJ, Martínez ME (2010). Colon Cancer Family Registry. Case-control study of

overweight, obesity, and colorectal cancer risk, overall and by tumor microsatellite instability status. J Natl Cancer Inst. 102(6): 391-400. doi: 10.1093/jnci/djq011.

16. Campbell PT, Newton CC, Freedman ND, Koshiol J, Alavanja MC, Beane Freeman LE, Buring JE, Chan AT, Chong DQ, Datta M, Gaudet MM, Gaziano JM, Giovannucci EL, Graubard BI, Hollenbeck AR, King L, Lee IM, Linet MS, Palmer JR, Petrick JL, Poynter JN, Purdue MP, Robien K, Rosenberg L, Sahasrabuddhe VV, Schairer C, Sesso HD, Sigurdson AJ, Stevens VL, Wactawski-Wende J, Zeleniuch-Jacquotte A, Renehan AG, McGlynn KA (2016). Body Mass Index, Waist Circumference, Diabetes, and Risk of Liver Cancer for U.S. Adults. Cancer Res. 76(20): 6076-6083. doi: 10.1158/0008-5472.CAN-16-0787.
17. Chooi YC, Ding C, Magkos F (2010). The epidemiology of obesity. Metabolism. 92: 6-10. doi: 10.1016/j.metabol.2018.09.005.
18. Chow WH, Blot WJ, Vaughan TL, Risch HA, Gammon MD, Stanford JL, Dubrow R, Schoenberg JB, Mayne ST, Farrow DC, Ahsan H, West AB, Rotterdam H, Niwa S, Fraumeni JF Jr (1998). Body mass index and risk of adenocarcinomas of the esophagus and gastric cardia. J Natl Cancer Inst. 90(2): 150-5. doi: 10.1093/jnci/90.2.150.
19. Corley DA, Kubo A, Zhao W (2008). Abdominal obesity and the risk of esophageal and gastric cardia carcinomas. Cancer Epidemiol Biomarkers Prev. 17(2): 352-8. doi: 10.1158/1055-9965.EPI-07-0748.
20. Correa P (2013). Gastric cancer: overview. GastroenterolClin North Am. 42(2): 211-7. doi: 10.1016/j.gtc.2013.01.002.
21. Crew KD, Neugut AI (2006). Epidemiology of gastric cancer. World J Gastroenterol. 12(3): 354-62. doi: 10.3748/wjg.v12.i3.354.
22. Csige I, Ujvárosy D, Szabó Z, Lőrincz I, Paragh G, Harangi M, Somodi S (2018). The Impact of Obesity on the Cardiovascular System. J Diabetes Res. 2018:

3407306. doi: 10.1155/2018/3407306.

23. De Pergola G, Silvestris F (2013). Obesity as a major risk factor for cancer. J Obes. 2013: 291546. doi: 10.1155/2013/291546.
24. Engeland A, Tretli S, Bjørge T (2004). Height and body mass index in relation to esophageal cancer; 23-year follow-up of two million Norwegian men and women. Cancer Causes Control. 15(8): 837-43. doi: 10.1023/B:CACO.0000043434.21558.ea.
25. Engeland A, Tretli S, Austad G, Bjørge T (2005). Height and body mass index in relation to colorectal and gallbladder cancer in two million Norwegian men and women. Cancer Causes Control. 16(8): 987-96. doi: 10.1007/s10552-005-3638-3.
26. Fan JH, Wang JB, Wang SM, Abnet CC, Qiao YL, Taylor PR (2017). Body mass index and risk of gastric cancer: A 30-year follow-up study in the Linxian general population trial cohort. Cancer Sci. 108(8): 1667-1672. doi: 10.1111/cas.13292. 2017 Jun 30.
27. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 136(5): E359-86. doi: 10.1002/ijc.29210.
28. Hagström H, Tynelius P, Rasmussen F (2018). High BMI in late adolescence predicts future severe liver disease and hepatocellular carcinoma: a national, population-based cohort study in 1.2 million men. Gut. 67(8): 1536-1542. doi: 10.1136/gutjnl-2016-313622.
29. Han X, Stevens J, Truesdale KP, Bradshaw PT, Kucharska-Newton A, Prizment AE, Platz EA, Joshu CE (2014). Body mass index at early adulthood, subsequent weight change and cancer incidence and mortality. Int J Cancer. 135(12): 2900-9. doi: 10.1002/ijc.28930.
30. Hartgrink HH, Jansen EP, van Grieken NC, van de Velde CJ (2009). Gastric cancer. Lancet. 374(9688): 477-90. doi: 10.1016/S0140-6736(09)60617-6.

31. Hashimoto Y, Hamaguchi M, Obora A, Kojima T, Fukui M (2020). Impact of metabolically healthy obesity on the risk of incident gastric cancer: a population-based cohort study. BMC EndocrDisord. 20(1): 11. doi: 10.1186/s12902-019-0472-2.
32. Hirabayashi M, Inoue M, Sawada N, Saito E, Abe SK, Hidaka A, Iwasaki M, Yamaji T, Shimazu T, Shibuya K, Tsugane S (2019). JPHC Study Group. Effect of body-mass index on the risk of gastric cancer: A population-based cohort study in A Japanese population. Cancer Epidemiol. 63: 101622. doi: 10.1016/j.canep.2019.101622.
33. Hughes LA, Simons CC, van den Brandt PA, Goldbohm RA, van Engeland M, Weijenberg MP (2011). Body size and colorectal cancer risk after 16.3 years of follow-up: an analysis from the Netherlands Cohort Study. Am J Epidemiol. 174(10): 1127-39. doi: 10.1093/aje/kwr247. 2011 Oct 7.
34. Ilic M, Ilic I (2022). Epidemiology of stomach cancer. World J Gastroenterol. 28(12): 1187. doi: 10.3748/wjg.v28.i12.1187.
35. Inoue M, Kurahashi N, Iwasaki M, Tanaka Y, Mizokami M, Noda M, Tsugane S (2009). Japan Public Health Center-based Prospective Study Group. Metabolic factors and subsequent risk of hepatocellular carcinoma by hepatitis virus infection status: a large-scale population-based cohort study of Japanese men and women (JPHC Study Cohort II). Cancer Causes Control. 20(5): 741-50. doi: 10.1007/s10552-008-9287-6.
36. Isaksson B, Jonsson F, Pedersen NL, Larsson J, Feychting M, Permert J (2002). Lifestyle factors and pancreatic cancer risk: a cohort study from the Swedish Twin Registry. Int J Cancer. 98(3): 480-2. doi: 10.1002/ijc.10256.
37. Jee SH, Yun JE, Park EJ, Cho ER, Park IS, Sull JW, Ohrr H, Samet JM (2008). Body mass index and cancer risk in Korean men and women. Int J Cancer. 123(8): 1892-6. doi: 10.1002/ijc.23719.
38. Jeong SH, Kim P, Yi SW, Kim YJ, Baeg MK, Yi JJ (2018). Body mass index and

gastrointestinal cancer mortality in Korean adults: A prospective cohort study. J GastroenterolHepatol. doi: 10.1111/jgh.14115.

39. Juo YY, Gibbons MAM, Dutson E, Lin AY, Yanagawa J, Hines OJ, Eibl G, Chen Y (2018). Obesity Is Associated with Early Onset of Gastrointestinal Cancers in California. J Obes. 2018: 7014073. doi: 10.1155/2018/7014073.
40. Karczewski J, Begier-Krasińska B, Staszewski R, Popławska E, Gulczynska-Elhadi K, Dobrowolska A (2019). Obesity and the Risk of Gastrointestinal Cancers. Dig Dis Sci. 64(10): 2740-2749. doi: 10.1007/s10620-019-05603-9. 2019 Apr 9.
41. Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD (2005). Cholangiocarcinoma. Lancet. 366(9493): 1303-14. doi: 10.1016/S0140-6736(05)67530-7.
42. Kim HJ, Kim N, Kim HY, Lee HS, Yoon H, Shin CM, Park YS, Park DJ, Kim HH, Lee KH, Kim YH, Kim HM, Lee DH (2015). Relationship between body mass index and the risk of early gastric cancer and dysplasia regardless of Helicobacter pylori infection. Gastric Cancer. 18(4): 762-73. doi: 10.1007/s10120-014-0429-0.
43. Klint A, Engholm G, Storm HH, Tryggvadóttir L, Gislum M, Hakulinen T, Bray F (2010). Trends in survival of patients diagnosed with cancer of the digestive organs in the Nordic countries 1964-2003 followed up to the end of 2006. ActaOncol. 49(5): 578-607. doi: 10.3109/02841861003739330.
44. Krupa-Kotara K, Dakowska D (2021). Impact of obesity on risk of cancer. Cent Eur J Public Health. 29(1):38-44. doi: 10.21101/cejph.a5913.
45. Lagergren J, Bergström R, Nyrén O (1999). Association between body mass and adenocarcinoma of the esophagus and gastric cardia. Ann Intern Med. 130(11): 883-90. doi: 10.7326/0003-4819-130-11-199906010-00003.
46. Larsson SC, Permert J, Håkansson N, Näslund I, Bergkvist L, Wolk A (2005). Overall obesity, abdominal adiposity, diabetes and cigarette smoking in relation to the risk of

pancreatic cancer in two Swedish population-based cohorts. Br J Cancer. 93(11): 1310-5. doi: 10.1038/sj.bjc.6602868.

47. Levi Z, Kark JD, Barchana M, Liphshitz I, Zavdy O, Tzur D, Derazne E, Furman M, Niv Y, Gordon B, Afek A, Shamiss A (2011). Measured body mass index in adolescence and the incidence of colorectal cancer in a cohort of 1.1 million males. Cancer Epidemiol Biomarkers Prev. 20(12): 2524-31. doi: 10.1158/1055-9965.EPI-11-0531.
48. Zohar L, Rottenberg Y, Twig G, Katz L, Leiba A, Derazne E, Tzur D, Eizenstein S, Keinan-Boker L, Afek A, Kark JD (2019). Adolescent overweight and obesity and the risk for pancreatic cancer among men and women: a nationwide study of 1.79 million Israeli adolescents. Cancer. 125(1): 118-126. doi: 10.1002/cncr.31764.
49. LiLi Y, Yatsuya H, Yamagishi K, Wakai K, Tamakoshi A, Iso H, Mori M, Sakauchi F, Motohashi Y, Tsuji I, Nakamura Y, Mikami H, Kurosawa M, Hoshiyama Y, Tanabe N, Tamakoshi K, Tokudome S, Suzuki K, Hashimoto S, Kikuchi S, Wada Y, Kawamura T, Watanabe Y, Ozasa K, Miki T, Date C, Sakata K, Kurozawa Y, Yoshimura T, Fujino Y, Shibata A, Okamoto N, Shio H (2013). Body mass index and weight change during adulthood are associated with increased mortality from liver cancer: the JACC Study. J Epidemiol. 23(3): 219-26. doi: 10.2188/jea.je20120199.
50. Lin Y, Kikuchi S, Tamakoshi A, Yagyu K, Obata Y, Inaba Y, Kurosawa M, Kawamura T, Motohashi Y, Ishibashi T (2007). JACC Study Group. Obesity, physical activity and the risk of pancreatic cancer in a large Japanese cohort. Int J Cancer. 120(12): 2665-71. doi: 10.1002/ijc.22614.
51. Lindblad M, Rodríguez LA, Lagergren J (2005). Body mass, tobacco and alcohol and risk of esophageal, gastric cardia, and gastric non-cardia adenocarcinoma among men and women in a nested case-control study. Cancer Causes Control.

16(3): 285-94. doi: 10.1007/s10552-004-3485-7.

52. Lindkvist B, Johansen D, Stocks T, Concin H, Bjørge T, Almquist M, Häggström C, Engeland A, Hallmans G, Nagel G, Jonsson H, Selmer R, Ulmer H, Tretli S, Stattin P, Manjer J (2014). Metabolic risk factors for esophageal squamous cell carcinoma and adenocarcinoma: a prospective study of 580,000 subjects within the Me-Can project. BMC Cancer. 14: 103. doi: 10.1186/1471-2407-14-103.
53. Liu CY, Chen KF, Chen PJ (2015). Treatment of Liver Cancer. Cold Spring HarbPerspect Med. 5(9): a021535. doi: 10.1101/cshperspect.a021535.
54. Luo J, Iwasaki M, Inoue M, Sasazuki S, Otani T, Ye W, Tsugane S (2007). JPHC Study Group. Body mass index, physical activity and the risk of pancreatic cancer in relation to smoking status and history of diabetes: a large-scale population-based cohort study in Japan--the JPHC study. Cancer Causes Control. 18(6): 603-12. doi: 10.1007/s10552-007-9002-z.
55. Luo J, Margolis KL, Adami HO, LaCroix A, Ye W (2008). Women's Health Initiative Investigators. Obesity and risk of pancreatic cancer among postmenopausal women: the Women's Health Initiative (United States). Br J Cancer. 99(3): 527-31. doi: 10.1038/sj.bjc.6604487.
56. MacInnis RJ, English DR, Hopper JL, Giles GG (2006). Body size and composition and the risk of gastric and oesophageal adenocarcinoma. Int J Cancer. 118(10): 2628-31. doi: 10.1002/ijc.21638.
57. Marley AR, Nan H (2016). Epidemiology of colorectal cancer. Int J MolEpidemiol Genet. 7(3): 105-114.
58. Mármol I, Sánchez-de-Diego C, PradillaDieste A, Cerrada E, Rodriguez Yoldi MJ (2017). Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer. Int J Mol Sci. 18(1): 197. doi: 10.3390/ijms18010197.
59. Matsuo K, Mizoue T, Tanaka K, Tsuji I, Sugawara Y, Sasazuki S, Nagata C, Tamakoshi A, Wakai K, Inoue M, Tsugane S (2012). Development and Evaluation of

Cancer Prevention Strategies in Japan. Association between body mass index and the colorectal cancer risk in Japan: pooled analysis of population-based cohort studies in Japan. Ann Oncol. 23(2): 479-90. doi: 10.1093/annonc/mdr143.

60. Merry AH, Schouten LJ, Goldbohm RA, van den Brandt PA (2007). Body mass index, height and risk of adenocarcinoma of the oesophagus and gastric cardia: a prospective cohort study. Gut. 56(11): 1503-11. doi: 10.1136/gut.2006.116665.
61. Michaud DS, Giovannucci E, Willett WC, Colditz GA, Stampfer MJ, Fuchs CS (2001). Physical activity, obesity, height, and the risk of pancreatic cancer. JAMA. 286(8): 921-9. doi: 10.1001/jama.286.8.921.
62. Brown LM, Swanson CA, Gridley G, Swanson GM, Schoenberg JB, Greenberg RS, Silverman DT, Pottern LM, Hayes RB, Schwartz AG (1995). Adenocarcinoma of the esophagus: role of obesity and diet. J Natl Cancer Inst. 87(2): 104-9. doi: 10.1093/jnci/87.2.104.
63. Ng SW, Popkin BM (2012). Time use and physical activity: a shift away from movement across the globe. Obes Rev. 13(8): 659-80. doi: 10.1111/j.1467-789X.2011.00982.x.
64. Nöthlings U, Wilkens LR, Murphy SP, Hankin JH, Henderson BE, Kolonel LN (2007). Body mass index and physical activity as risk factors for pancreatic cancer: the Multiethnic Cohort Study. Cancer Causes Control. 18(2): 165-75. doi: 10.1007/s10552-006-0100-0.
65. Odegaard AO, Koh WP, Yu MC, Yuan JM (2011). Body mass index and risk of colorectal cancer in Chinese Singaporeans: the Singapore Chinese Health Study. Cancer. 117(16): 3841-9. doi: 10.1002/cncr.25936. 2011 Feb 24.
66. O'Doherty MG, Freedman ND, Hollenbeck AR, Schatzkin A, Abnet CC (2012). A prospective cohort study of obesity and risk of oesophageal and gastric adenocarcinoma in the NIH-AARP Diet and Health Study. Gut. 61(9): 1261-8. doi: 10.1136/gutjnl-2011-300551.

67. Oh SW, Yoon YS, Shin SA (2005). Effects of excess weight on cancer incidences depending on cancer sites and histologic findings among men: Korea National Health Insurance Corporation Study. J ClinOncol. 23(21): 4742-54. doi: 10.1200/JCO.2005.11.726.
68. Ohki T, Tateishi R, Sato T, Masuzaki R, Imamura J, Goto T, Yamashiki N, Yoshida H, Kanai F, Kato N, Shiina S, Yoshida H, Kawabe T, Omata M (2008). Obesity is an independent risk factor for hepatocellular carcinoma development in chronic hepatitis C patients. ClinGastroenterolHepatol. 6(4): 459-64. doi: 10.1016/j.cgh.2008.02.012.
69. Oxentenko AS, Bardia A, Vierkant RA, Wang AH, Anderson KE, Campbell PT, Sellers TA, Folsom AR, Cerhan JR, Limburg PJ (2010). Body size and incident colorectal cancer: a prospective study of older women. Cancer Prev Res (Phila). 3(12): 1608-20. doi: 10.1158/1940-6207.CAPR-10-0116. 2010 Aug 18.
70. Pan SY, Johnson KC, Ugnat AM, Wen SW, Mao Y (2004). Canadian Cancer Registries Epidemiology Research Group. Association of obesity and cancer risk in Canada. Am J Epidemiol. 159(3): 259-68. doi: 10.1093/aje/kwh041.
71. Park SL, Lee YC, Marron M, Agudo A, Ahrens W, Barzan L, Bencko V, Benhamou S, Bouchardy C, Canova C, Castellsague X, Conway DI, Healy CM, Holcátová I, Kjaerheim K, Lagiou P, Lowry RJ, Macfarlane TV, Macfarlane GJ, McCartan BE, McKinney PA, Merletti F, Pohlabeln H, Richiardi L, Simonato L, Sneddon L, Talamini R, Trichopoulos D, Znaor A, Brennan P, Hashibe M (2011). The association between change in body mass index and upper aerodigestive tract cancers in the ARCAGE project: multicenter case-control study. Int J Cancer. 128(6): 1449-61. doi: 10.1002/ijc.25468.
72. Patel AV, Rodriguez C, Bernstein L, Chao A, Thun MJ, Calle EE (2005). Obesity, recreational physical activity, and risk of pancreatic cancer in a large U.S. Cohort. Cancer Epidemiol Biomarkers Prev.

14(2): 459-66. doi: 10.1158/1055-9965.EPI-04-0583.

73. Polesel J, Zucchetto A, Montella M, Dal Maso L, Crispo A, La Vecchia C, Serraino D, Franceschi S, Talamini R (2009). The impact of obesity and diabetes mellitus on the risk of hepatocellular carcinoma. Ann Oncol. 20(2): 353-7. doi: 10.1093/annonc/mdn565.
74. Prasad S, Tyagi AK (2015). Ginger and its constituents: role in prevention and treatment of gastrointestinal cancer. Gastroenterol Res Pract. 2015: 142979. doi: 10.1155/2015/142979.
75. Rapp K, Schroeder J, Klenk J, Stoehr S, Ulmer H, Concin H, Diem G, Oberaigner W, Weiland SK (2005). Obesity and incidence of cancer: a large cohort study of over 145,000 adults in Austria. Br J Cancer. 93(9): 1062-7. doi: 10.1038/sj.bjc.6602819.
76. Ryan AM, Rowley SP, Fitzgerald AP, Ravi N, Reynolds JV (2006). Adenocarcinoma of the oesophagus and gastric cardia: male preponderance in association with obesity. Eur J Cancer. 42(8): 1151-8. doi: 10.1016/j.ejca.2005.12.024.
77. Samanic C, Chow WH, Gridley G, Jarvholm B, Fraumeni JF Jr (2006). Relation of body mass index to cancer risk in 362,552 Swedish men. Cancer Causes Control. 17(7): 901-9. doi: 10.1007/s10552-006-0023-9.
78. Schlesinger S, Aleksandrova K, Pischon T, Fedirko V, Jenab M, Trepo E, Boffetta P, Dahm CC, Overvad K, Tjønneland A, Halkjær J, Fagherazzi G, Boutron-Ruault MC, Carbonnel F, Kaaks R, Lukanova A, Boeing H, Trichopoulou A, Bamia C, Lagiou P, Palli D, Grioni S, Panico S, Tumino R, Vineis P, Bueno-de-Mesquita HB, van den Berg S, Peeters PH, Braaten T, Weiderpass E, Quirós JR, Travier N, Sánchez MJ, Navarro C, Barricarte A, Dorronsoro M, Lindkvist B, Regner S, Werner M, Sund M, Khaw KT, Wareham N, Travis RC, Norat T, Wark PA, Riboli E, Nöthlings U (2013). Abdominal obesity, weight gain during adulthood and risk of liver and biliary tract cancer in a European cohort. Int J Cancer. 132(3): 645-57. doi: 10.1002/ijc.27645.

79. Setiawan VW, Lim U, Lipworth L, Lu SC, Shepherd J, Ernst T, Wilkens LR, Henderson BE, Le Marchand L (2016). Sex and Ethnic Differences in the Association of Obesity With Risk of Hepatocellular Carcinoma. ClinGastroenterolHepatol. 14(2): 309-16. doi: 10.1016/j.cgh.2015.09.015.
80. Siegel RL, Miller KD, Jemal A (2017). Cancer Statistics, 2017. CA Cancer J Clin. 67(1): 7-30. doi: 10.3322/caac.21387. 2017 Jan 5.
81. Smyth EC, Lagergren J, Fitzgerald RC, Lordick F, Shah MA, Lagergren P, Cunningham D (2017). Oesophageal cancer. Nat Rev Dis Primers. 3: 17048. doi: 10.1038/nrdp.2017.48.
82. Song H, Saito E, Sawada N, Abe SK, Hidaka A, Shimazu T, Yamaji T, Goto A, Iwasaki M, Sasazuki S, Ye W, Inoue M, Tsugane S (2017). Body mass index change during adulthood and risk of oesophageal squamous-cell carcinoma in a Japanese population: the Japan Public Health (JPHC)-based prospective study. Br J Cancer. 117(11): 1715-1722. doi: 10.1038/bjc.2017.332. 2017 Sep 26.
83. Song M, Choi JY, Yang JJ, Sung H, Lee Y, Lee HW, Kong SH, Lee HJ, Kim HH, Kim SG, Yang HK, Kang D (2015). Obesity at adolescence and gastric cancer risk. Cancer Causes Control. 26(2): 247-256. doi: 10.1007/s10552-014-0506-z.
84. Steffen A, Huerta JM, Weiderpass E, Bueno-de-Mesquita HB, May AM, Siersema PD, Kaaks R, Neamat-Allah J, Pala V, Panico S, Saieva C, Tumino R, Naccarati A, Dorronsoro M, Sánchez-Cantalejo E, Ardanaz E, Quirós JR, Ohlsson B, Johansson M, Wallner B, Overvad K, Halkjaer J, Tjønneland A, Fagherazzi G, Racine A, Clavel-Chapelon F, Key TJ, Khaw KT, Wareham N, Lagiou P, Bamia C, Trichopoulou A, Ferrari P, Freisling H, Lu Y, Riboli E, Cross AJ, Gonzalez CA, Boeing H (2015). General and abdominal obesity and risk of esophageal and gastric adenocarcinoma in the European Prospective Investigation into Cancer and Nutrition. Int J

Cancer. 137(3): 646-57. doi: 10.1002/ijc.29432.

85. Stolzenberg-Solomon RZ, Adams K, Leitzmann M, Schairer C, Michaud DS, Hollenbeck A, Schatzkin A, Silverman DT (2008). Adiposity, physical activity, and pancreatic cancer in the National Institutes of Health-AARP Diet and Health Cohort. Am J Epidemiol. 167(5): 586-97. doi: 10.1093/aje/kwm361.
86. Stolzenberg-Solomon RZ, Schairer C, Moore S, Hollenbeck A, Silverman DT (2013). Lifetime adiposity and risk of pancreatic cancer in the NIH-AARP Diet and Health Study cohort. Am J ClinNutr. 98(4): 1057-65. doi: 10.3945/ajcn.113.058123.
87. Swinburn BA, Sacks G, Hall KD, McPherson K, Finegood DT, Moodie ML, Gortmaker SL (2011). The global obesity pandemic: shaped by global drivers and local environments. Lancet. 378(9793): 804-14. doi: 10.1016/S0140-6736(11)60813-1.
88. Thrift AP, Whiteman DC (2012). The incidence of esophageal adenocarcinoma continues to rise: analysis of period and birth cohort effects on recent trends. Ann Oncol. 23(12): 3155-3162. doi: 10.1093/annonc/mds181.
89. Upadhyay J, Farr O, Perakakis N, Ghaly W, Mantzoros C (2018). Obesity as a Disease. Med Clin North Am. 102(1): 13-33. doi: 10.1016/j.mcna.2017.08.004.
90. Valastyan S, Weinberg RA (2011). Tumor metastasis: molecular insights and evolving paradigms. Cell. 147(2): 275-92. doi: 10.1016/j.cell.2011.09.024.
91. Van Cutsem E, Sagaert X, Topal B, Haustermans K, Prenen H (2016). Gastric cancer. Lancet. 388(10060): 2654-2664. doi: 10.1016/S0140-6736(16)30354-3.
92. Veugelers PJ, Porter GA, Guernsey DL, Casson AG (2006). Obesity and lifestyle risk factors for gastroesophageal reflux disease, Barrett esophagus and esophageal adenocarcinoma. Dis Esophagus. 19(5): 321-8. doi: 10.1111/j.1442-2050.2006.00602.x.
93. Whiteman DC, Sadeghi S, Pandeya N, Smithers BM, Gotley DC, Bain CJ, Webb PM, Green AC (2008). Australian

Cancer Study. Combined effects of obesity, acid reflux and smoking on the risk of adenocarcinomas of the oesophagus. Gut. 57(2): 173-80. doi: 10.1136/gut.2007.131375.

94. World Health Organization. Obesity and overweight. Fact sheet no 311 January 2015.
95. World Cancer Research Fund/American Institute for Cancer Research. Continious Update Project Expert Report 2018, Body fatness and weight gain and the risk of cancer, London, the United Kingdom, 2018.
96. Yamaoka Y, Kato M, Asaka M (2008). Geographic differences in gastric cancer incidence can be explained by differences between Helicobacter pylori strains. Intern Med. 47(12): 1077-83. doi: 10.2169/internalmedicine.47.0975.
97. Yang B, Petrick JL, Kelly SP, Graubard BI, Freedman ND, McGlynn KA (2017). Adiposity across the adult life course and incidence of primary liver cancer: The NIH-AARP cohort. Int J Cancer. 141(2): 271-278. doi: 10.1002/ijc.30737.
98. Xie Y, Shi L, He X, Luo Y (2021). Gastrointestinal cancers in China, the USA, and Europe. Gastroenterol Rep (Oxf). 9(2): 91-104. doi: 10.1093/gastro/goab010.
99. Zohar L, Rottenberg Y, Twig G, Katz L, Leiba A, Derazne E, Tzur D, Eizenstein S, Keinan-Boker L, Afek A, Kark JD (2019). Adolescent overweight and obesity and the risk for pancreatic cancer among men and women: a nationwide study of 1.79 million Israeli adolescents. Cancer. 125(1): 118-126. doi: 10.1002/cncr.31764.

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Impact of COVID-19 in Diabetes, Asthma, and Cardiovascular Diseases

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ABSTRACT: *SARS-CoV-2 a RNA virus, is a novel virus that belongs to the Coronaviridae family and has caused the most lethal pandemic of the current century. Various variants of SARS-CoV-2 have been circulating since the start of the COVID-19 outbreak. Its variants are Alpha, Beta, Gamma, Delt, Mu and Omicron. The fusion of the viral envelope and host membrane occurs when the spike protein of the virus interacts with the host's ACE2 receptor, resulting in the release of viral RNA into the cytoplasm of the host cell. This process is a crucial step in the viral replication cycle, as it allows the virus to hijack the host cell's machinery to produce more copies of itself. Post-COVID-19 complications such as reduced blood flow to the pancreas, myocardial damage and elevated blood clotting levels have been observed. Studies have shown that individuals with obesity, diabetes, and hypertension are more susceptible to contracting COVID-19. There is still an ongoing debate among experts regarding the impact of asthma as a premorbid condition on the course of the disease caused by SARS-CoV-2. The goal of this review is to give a general description of SARS-CoV-2 and highlight COVID-19's potentially negative effects on health.*

Keywords: *SARS-CoV-2, COVID-19, Variants of SARS-Cov-2, POST COVID-19 Complications*

INTRODUCTION

An increasing number of individuals are recovering from the disease but still dealing with its after effects after their initial infection has subsided as the COVID-19 pandemic continues to grip the world (Carlsten et al., 2021). These lingering symptoms, sometimes known as "long COVID" or "post-COVID syndrome," can last for weeks

or even months and have a major impact on the affected person's quality of life. Little is known about the wide spectrum of post-COVID-19 health manifestations that might affect different organs and systems in the body, despite the fact that the respiratory symptoms of COVID-19 are well-documented (Vu and McGill, 2021). Many of the most often reported post-COVID-19 health symptoms are briefly reviewed in this article, along with their possible causes while the data was gathered from several articles. In order to create efficient post-COVID-19 care methods and enhance the outcomes for persons impacted by this novel disease, healthcare practitioners and policy makers must have a thorough understanding of these symptoms.

SARS-CoV-2 Genotype

A new type of coronavirus known as SARS-CoV-2 caused a sudden outbreak of viral pneumonia in Wuhan, China, which then quickly spread to become a pandemic (Yasmeen et al., 2021; Yasmeen and Chaudhry, 2022). Based upon the phylogenetic relationships of genotypic structure, COVID-19 belongs to the genera "Betacoronavirus". In humans, Betacoronaviruses (SARS-CoV-2, SARS-CoV, and MERS-CoV) have various similarities. Still, their genomic and phenotypic structures already have some different features that may affect their pathogenesis. It is a single-stranded RNA (ssRNA) negative sense particle (Chen et al., 2020).

Variants of SARS-CoV-2

All types of viruses, including SARS-CoV-2, COVID-19 progress over time and change their properties. The term "variant" is utilized to refer to a subtype of a virus that possesses genetic differences from the main strain, but these differences are not significant enough to categorize it as a separate strain. Depending on the changes in the virus's genetic material, mutations may affect coronavirus properties such as transmission or strength (Chen et al., 2020).

Various variants of corona SARS-Cov-2 that are Alpha (the earliest recorded samples in Sep-2020), Beta (the

earliest recorded samples in May-2020), Gamma (the earliest recorded samples in Nov-2020), Delta (the earliest recorded samples in Oct-2020), Mu (Being Monitored:Sep-2021), R.1 Omicron (the earliest recorded samples in Nov-2021) and shown in (Fig. 1).

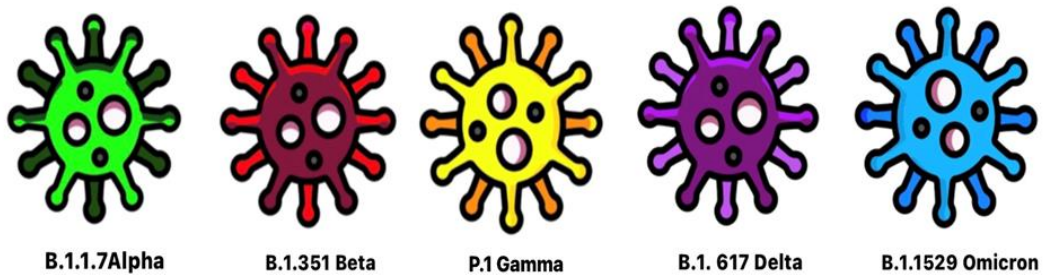

Fig. 1. Various variants SARS-CoV-2 (adopted by Chrysostomou et al., 2023)

Lifecycle and genomic structure of SARS-CoV-2

SARS-CoV-2 is a spherical, enveloped virus that has single-stranded negative sense RNA. SARS-CoV-2 consists of four structural proteins i.e., Spike protein, membrane protein, envelope protein, and nucleocapsid protein. The spike protein binds to the host cell to begin the early stage of the virus life cycle. The initial phase of the virus life cycle begins with entrance of virus into the host cell after the spike protein binds to the ACE2 receptor on the host cell (. SARS-CoV-2 entrance occurs either by endocytosis or through direct fusion. The action of cathepsin L in lysosomes seems to stimulate further processing, which eventually promotes the fusion of the viral envelope with the host membrane and the release of the viral RNA (De Haan et al., 1998). Then the Virus hijacks the biosynthetic machinery of the host cell and starts viral replication. Viral copies are released with bursting of the host cell and cause severe respiratory infection in the host's body.

POST COVID-19 Implications

1. Diabetes Mellitus

COVID-19 is a global pandemic that continues to pose unprecedented challenges worldwide. It is caused by enveloped RNA beta-coronavirus

(SARS-CoV-2) (Viswanathan et al., 2021). Pakistan, Indonesia, Mexico, India, the United States of America, Brazil, and Bangladesh are the top seven countries with the highest prevalence of diabetes. They are the top 8% contributors to coronavirus deaths worldwide.

This may significantly impact COVID-19-related mortality (Viswanathan et al., 2021). It was also observed that some patients having no previous history of diabetes developed Diabetic ketoacidosis (DKA) when exposed to SARS-CoV-2(Bhutani and Bhutani, 2014; Blind et al., 2018).

SARS-CoV-2 acts on the ACE2 receptors and gets entry into the pancreatic cells. By using ACE2 immunostaining of pancreas tissue, an interesting study conducted by Thaweerat et al. (Thaweerat, 2020) revealed that the endocrine part of the pancreas surprisingly has more ACE2 receptors as compared to its exocrine one (Thaweerat, 2020). The endocrine region of the pancreas control the blood glucose levels (Roder et al., 2016). SARS-CoV-2 invades the pancreas and the immune system, and destroys pancreatic cells lowering insulin production, thus resulting in DM, which is the most common in severe cases of COVID-19 (Kamrath et al., 2020; Liu et al., 2020; Marchand et al., 2020; Oriot & Hermans, 2022). This process is activated because of SARS-CoV2 infections, leading to the formation of antibodies in pancreatic cells (Op de Beeck and Eizirik, 2016).

The hypothesis linking DM-COVID-19 suggests that the coronavirus may harm the islet cells in the pancreas, leading to impaired insulin levels and potentially causing diabetes (Liu et al., 2020; Thaweerat, 2020).

This pathway is also mentioned in ifferent studies (Baracchini et al., 2020; Mota and Stefan, 2020) in their work on COVID-19.

In addition, endocytosis of COVID-19 lowers the level of ACE2, leading to increased levels of Angiotensin II. Angiotensin II is a potent vasoconstrictor which inhibits action of nitric oxide in the endothelium of the islet capillaries (Balasubramanyam, 2020), and decreased the blood supply

to the pancreas. Islet cells comprised 15 percent blood supply to the pancreas and now makeup only 1 to 2 percent of the pancreatic volume (Jansson and Hellerström, 1983). The decrease in blood flow to islet cells caused by vasoconstriction hinders insulin secretion in the pancreas. This confirms the bidirectional relationship between COVID-19 and diabetes (Carlsson et al., 1998). Barron et al. claimed that the entire population of England has both type 1 and type 2 diabetes. According to a survey conducted in the USA, people with diabetes mellitus are more likely to experience other consequences of COVID-19 (Zhang et al., 2013). Diabetes related SARS-CoV-2 pathway is also shown in Fig. 2

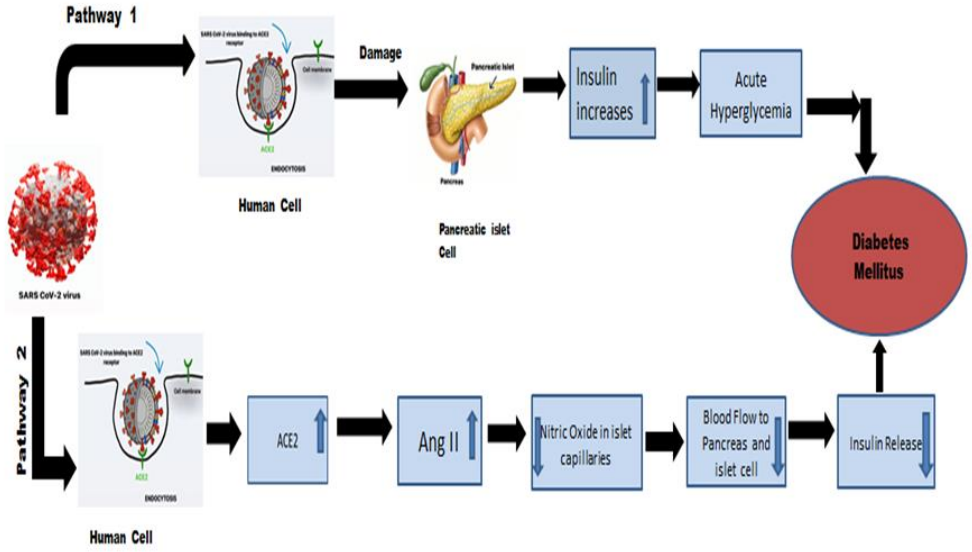

Fig. 2. Diabetes related SARS-CoV-2 pathway (adopted by www.fda.gov)

2. Cardiovascular Diseases

The emergence of the SARS-CoV-2 has presented an unequal challenge to the global health community. The rapid spread of the coronavirus and the resulting epidemics were facilitated by the potential for infection during the asymptomatic phase. Heart disease and various other factors (age, male sex, current smoking, hypertension, and diabetes) increased incidence of coronavirus (Wu and McGoogan, 2020). According to research, SARS-CoV-2 can worsen underlying

cardiovascular disease and cause acute heart problems. Although SARS-CoV-2 main target is the respiratory tract, it may also affect the cardiovascular system. The following are typical routes by which SARS-CoV-2 patients experience cardiovascular issues (Li et al., 2020; Xiong et al., 2020).

1. SARS-CoV-2 causes direct myocardial injury. The virus enters human cells through ACE2. They are abundant within the heart and pancreas. ACE2 regulates the neurohumoral role of the cardiovascular system in both normal and disease conditions. SARS-CoV-2 is attached to ACE2 and can alter the signalling pathways of ACE2, which may cause an acute myocardial injury (Li et al., 2020; Xiong et al., 2020; Yasmeen et al., 2021).
2. Severe cases of SARS-CoV-2 are usually characterized by systemic inflammation (SI) that combined with less oxygen can cause an acute respiratory illness that impairs myocardial oxygen demand and supply relations, leading to myocardial injury.
3. The usage of antiviral drugs, corticosteroids, and other medicines for treating COVID-19 may negatively affect the cardiovascular system.
4. An increase in coronary blood flow causes inflammation, resulting in rupture of coronary artery thrombosis, and causing acute myocardial infarction. Systemic inflammation causes a prothrombotic milieu that further increases the cardiovascular disease risk.
5. Acute myocardial injury (AMI) is the most common cardiovascular complication observed in viral infections. AMI is the death of cardiomyocytes manifested by an increased level of cardiac troponin I (cTnI). Although various cases are related to acute cardiac injury, approximately 7-11% of the positive cases have high cTnI levels (Lippi & Plebani, 2020). The meta-analysis of the Chinese research (B. Li et al., 2020)

claimed 8% of cases of acute cardiac injury; on the other hand, other studies include only patients who had definite outcomes meaning either death or discharge. Discharge from hospitals have shown 16.5% incidence of cTnI level (Zhou et al., 2020). Consistently, AMI has acted as a negative powerful prognostic marker in COVID-19 patients regardless of the actual cases (Huang et al., 2020; Wang et al., 2020; Zhou et al., 2020). Patients admitted to the ICU due to serious illness have a systematic risk of high troponin levels. In contrast, high troponin incidence was significantly lower in patients with minor illnesses that did not require ICU admission. In Pakistan, one in every seven children who develop moderate to severe COVID-19 dies, and the mortality rate is substantially higher than in Western nations.

3. Thrombosis

COVID-19 has affected more than 1/3rd of patients worldwide, as a result of affliction with high blood clotting levels. Even the first ninety nine covid patients admitted to hospitals in China show high D-dimer levels in the blood. Autopsies of people who died due to COVID-19 had shown widespread clots. Disseminated intravascular coagulation and thrombosis are two very common problems in SARS-CoV-2 infection. The blood clot has consumptive nature that lowers the concentration in the circulation of blood. Deletion of the clotting factors accounts for continued bleeding. Clotting results in rupturing of vessels and hemorrhagic stroke. In exuberant clotting, an anticoagulant protein, PROS1 acts with an active protein C to degrade Va and VIIIa factors, terminating the clots. In the coagulation cascade, it is an important inhibitory gene. The absence of PROS1 within mice are lethal, resulting in catastrophic blood clotting (CBC) and hemorrhage. Development

of immune hyper-reaction worsens COVID-19 patients' health. PROS1 is an anticoagulant in the blood coagulation cascade series. The high levels of TNF, IL-1, IL-6, and Interferon-γ concentration dramatically damage multiple sites pleiotropically. The researcher believed that PROS1 has a link with immune hyper-reaction and blood clotting. PROS1 is also the activating ligand of the TAM (Tyro3, Axl, and Mer) family of Tyrosine kinases receptors (RTKs) (Lemke, 2013). RTKs show macrophages, dendritic cells, and other immune bodies in the immune system. MER (Membrane estrogen receptor) is a kinase. When both TAM activating ligands, PROS1 and Gas6, bind to the extracellular domain of MER, MER signaling is activated that reduces type 1 interferons such as TNF and production of other cytokines (Lemke, 2013; Rothlin et al., 2007). A decrease in TAM receptor signals also decrease PROS1 expression.

4. Asthma

Asthma has become a serious health challenge for people 18-50 years old (Network, 2014). Every year more than 12 million people get affected by asthma in the United States (Fergeson et al., 2017). It is an infectious disease caused by COVID-19. The severity of coronavirus can range from mild to severe (Li et al., 2020). The effects of COVID-19 on asthma patients are less proven (Johnston, 2020). Conversely, the impact of coronavirus is closely linked to age.

In Castilla La-Mancha (region of Spain), clinical data of about 2,034,921 patients was collected. The data was collected from emergency wards, inpatient and outpatient units, and primary care centers. Approximately 71,182 asthma cases were reported from January 1, 2019, to May 10, 2020. It was observed that bronchial-asthma patients infected with SARS-CoV-2 were mainly old females and had high rates of obesity, diabetes mellitus, and hypertension compared to the asthmatic patients without COVID-19. Asthma patients using inhaled corticosteroids showed lower hospital admissions. Pneumonia with the variability of radiological

expressions was the most common diagnosis in hospitalized patients. This study concluded that the incidence of SARS-CoV-2 infection in asthmatic patients was low but slightly higher in the non-asthmatic general population. Old age and related comorbidities, for example, DM and CVD, act as increased risk factors in the hospitalization of asthmatic patients with COVID-19. Whether bronchitis or asthma is an independent risk factor in COVID-19 cases is less clear. It was found that the number of COVID-19-related hospital admissions and mortalities in asthmatic patients was surprisingly low.

The impact of the COVID-19 pandemic on healthcare systems around the world has been disastrous, but children appeared relatively safe. Asthmatic children do not appear to be disproportionately affected by SARS-CoV2. There is a risk that accesses to health care, treatment, and disease control will be restricted if the pandemic persists, particularly in the poorest states. Medical care models such as telemedicine, valid questionnaires and monitoring, and cloud technology usage adoption can help improve the management of chronic respiratory diseases such as asthma. In Korea, clinical data collected from approximately 7591 COVID-19 patients reported that the mortality rate (7.8%) for coronavirus patients with asthma was quite high compared to other patients (Guan et al., 2020).

COVID-19 and severe asthma data are scary. Recently, the Belgian Severe Asthma Registry published an article. This study reported that severe asthma does not act as an increased risk factor for COVID-19 infection (Hanon et al., 2020). No doubt asthma is a highly chronic disease that has affected approximately 4.4 percent of the world's population. Asthmatic exacerbations are stimulated by respiratory viruses, increasing the infectious condition's severity (Zheng et al., 2018). Coronaviruses had triggered asthma exacerbations in the past. However, for the novel coronavirus, it is still a controversy among researchers regarding the role

of SARS-CoV-2 in asthma as a premorbid that whether it worsens the progression of the disease (Caminati et al., 2020; Richardson et al., 2020).

CONCLUSION

According to a review of the cases of COVID-19 that have been documented, people with obesity, diabetes, and hypertension are more likely to have the disease. ACE2 receptors on the pancreas and heart allow coronavirus to enter, which damages the organ, lowers blood insulin levels, and leads to diabetes and myocardial injury. Thrombosis and diffuse intravascular coagulation, which reduces PROS1 expression can lead to auto-immune disease in SARS-CoV-2 patients. Regarding the SARS-CoV-2 role of asthma as a premorbid that affects the course of the disease, there is still debate among experts in this area.

REFERENCES

1. Balasubramanyam M (2020). Does COVID-19 Warn Us to Revisit Virus-Induced Diabetes? Expl. Res. Hypo. Med. 5(4): 129-133.
2. Baracchini C, Pieroni A, Kneihsl M, Azevedo E, Diomedi M, Pascazio L, Wojczal J, Lucas C, Bartels E, Bornstein N (2020). Practice recommendations for neurovascular ultrasound investigations of acute stroke patients in the setting of the COVID-19 pandemic: an expert consensus from the European Society of Neurosonology and Cerebral Hemodynamics. Eur. J. Neurol. 27(9): 1776-1780.
3. Bhutani J, Bhutani S (2014). Worldwide burden of diabetes. Ind. J. Endocrinol. Metabol. 18(6): 868.
4. Blind E, Janssen H, Dunder K, de Graeff PA (2018). The European Medicines Agency's approval of new medicines for type 2 diabetes. Diabe. Obes. Metabol. 20(9): 2059-2063.
5. Caminati M, Lombardi C, Micheletto C, Roca E, Bigni B, Furci F, Girelli D, Senna G, Crisafulli E (2020). Asthmatic patients in COVID-19 outbreak: few cases despite many cases. J.

Allerg Clin. Immunol. 146(3): 541-542.

6. Carlsson PO, Berne C, Jansson L (1998). Angiotensin II and the endocrine pancreas: effects on islet blood flow and insulin secretion in rats. Diabetol. 41(2): 127-133.
7. Carlsten C, Gulati M, Hines S, Rose C, Scott K, Tarlo SM, Torén K, Sood A, de la Hoz RE (2021). COVID-19 as an occupational disease. Am. J. Ind. Med. 64(4): 227-237.
8. Chen Y, Liu Q, Guo D (2020). Emerging coronaviruses: genome structure, replication, and pathogenesis. J. Med. Virol. 92(4): 418-423.
9. Chrysostomou AC, Aristokleous A, Rodosthenous JH, Christodoulou C, Stathi G, Kostrikis LG (2023). Detection of Circulating SARS-CoV-2 Variants of Concern (VOCs) Using a Multiallelic Spectral Genotyping Assay. Life. 13(2): 304.
10. De Haan CA, Kuo L, Masters PS, Vennema H, Rottier PJ (1998). Coronavirus particle assembly: primary structure requirements of the membrane protein. J. Virol. 72(8): 6838-6850.
11. Fergeson JE, Patel SS, Lockey RF (2017). Acute asthma, prognosis, and treatment. J. Allerg. Clin. Immunol. 139(2): 438-447.
12. Guan WJ, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM, Liu XQ, Chen RC, Tang CL, Wang T, Ou CQ (2020). Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. Europ. Resp. J. 55(5).
13. Hanon S, Brusselle G, Deschampheleire M, Louis R, Michils A, Peché R, Pilette C, Rummens P, Schuermans D, Simonis H (2020). COVID-19 and biologics in severe asthma: data from the Belgian Severe Asthma Registry. Europ. Respir. J. 56(6).
14. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet. 395(10223): 497-506.

15. Jansson L, Hellerström C (1983). Stimulation by glucose of the blood flow to the pancreatic islets of the rat. Diabetol. 25(1): 45-50.
16. Johnston SL (2020). Asthma and COVID-19: is asthma a risk factor for severe outcomes? Allergy. 75(7): 1543-1545.
17. Kamrath C, Mönkemöller K, Biester T, Rohrer TR, Warncke K, Hammersen J, Holl RW (2020). Ketoacidosis in children and adolescents with newly diagnosed type 1 diabetes during the COVID-19 pandemic in Germany. Jama. 324(8): 801-804.
18. Lemke G (2013). Biology of the TAM receptors. Cold Spring Harbor Perspec. Biol. 5(11): a009076.
19. Li B, Yang J, Zhao F, Zhi L, Wang X, Liu L, Bi Z, Zhao Y (2020). Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. Clin. Res. Cardiol. 109(5): 531-538.
20. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KS, Lau EH, Wong JY, Xing X (2020). Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. New Engl. J. Med.
21. Lippi G, Plebani M (2020). Laboratory abnormalities in patients with COVID-2019 infection. Clin. Chem. Lab. Med. 58(7): 1131-1134.
22. Liu F, Long X, Zhang B, Zhang W, Chen X, Zhang Z (2020). ACE2 expression in pancreas may cause pancreatic damage after SARS-CoV-2 infection. Clin. Gastroenterol. Hepatol. 18(9): 2128-2130. e2122.
23. Marchand L, Pecquet M, Luyton C (2020). Type 1 diabetes onset triggered by COVID-19. Acta diabetologica, 57(10): 1265-1266.
24. Mota M, Stefan AG (2020). Covid-19 and Diabetes–A Bidirectional Relationship? Romanian J. Diabet. Nut. Metabolic Dis. 27(2): 77-79.
25. Network GA (2014). Global Asthma Network. The Glob. Asthma Rep.
26. Op de Beeck A, Eizirik DL (2016). Viral infections in type 1 diabetes

mellitus—why the β cells? Nat. Rev. Endocrinol. 12(5): 263-273.

27. Oriot P, Hermans MP (2022). Euglycemic diabetic ketoacidosis in a patient with type 1 diabetes and SARS-CoV-2 pneumonia: case-report and review of the literature. Acta Clinica Belgica. 77(1): 113-117.
28. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, Barnaby DP, Becker LB, Chelico JD, Cohen SL, Cookingham J (2020). Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. Jama. 323(20): 2052-2059.
29. Roder PV, Wu B, Liu Y, Han W (2016). Pancreatic regulation of glucose homeostasis. Exp. Mol. Med. 48(1): 1-19.
30. Rothlin CV, Ghosh S, Zuniga EI, Oldstone MB, Lemke G (2007). TAM receptors are pleiotropic inhibitors of the innate immune response. Cell. 131(6): 1124-1136.
31. Thaweerat W (2020). Current evidence on pancreatic involvement in SARS-CoV-2 infection. Pancreatol. 20(5): 1013.
32. Viswanathan V, Puvvula A, Jamthikar AD, Saba L, Johri A M, Kotsis V, Khanna NN, Dhanjil SK, Majhail M, Misra DP (2021). Bidirectional link between diabetes mellitus and coronavirus disease 2019 leading to cardiovascular disease: A narrative review. World J. Diabetes. 12(3): 215.
33. Vu T, McGill SC (2021). An overview of post–covid-19 condition (long covid). Canadian J. Health Technol. 1(9).
34. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. Jama. 323(11): 1061-1069.
35. Wu Z, McGoogan JM (2020). Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19)

outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. Jama. 323(13): 1239-1242.

36. Xiong TY, Redwood S, Prendergast B, Chen M (2020). Coronaviruses and the cardiovascular system: acute and long-term implications. Europ. Heart J.
37. Zhang W, Li C, Liu B, Wu R, Zou N, Xu YZ, Yang YY, Zhang F, Zhou HM, Wan KQ (2013). Pioglitazone upregulates hepatic angiotensin converting enzyme 2 expression in rats with steatohepatitis. Ann. Hepatol. 12(6): 892-900.
38. Yasmeen R, Sunmmia K, Chaudhry S (2021). Systematic Review on Mechanism of Action, Mortalities, Morbidities, and Prevention from Covid-19. Pak. J. Sci. 2021; 73(2).
39. Yasmeen R, Chaudhry S (2022). Origin, Symptoms, Transmission and Preclusions of COVID-19. Pak. J. Zool. 54(4).
40. Yasmeen R, Mobeen N, Chaudhry S (2021). COVID-19 and Cardiovascular Diseases. Pak. J. Zool. 53(5).
41. Zheng XY, Xu YJ, Guan WJ, Lin LF (2018). Regional, age and respiratory-secretion-specific prevalence of respiratory viruses associated with asthma exacerbation: a literature review. Arch. Virol. 163(4): 845-853.
42. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The lancet. 395(10229): 1054-1062.

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Cochlear Implantation and Assessment of Speech in Children

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ABSTRACT: *Cochlea is a hollow, spiral shaped bone in the inner ear that has sense of hearing and to produce sound. Cochlear problems, or its damage can result in loss of hearing. This study was designed to access intelligibility of speech in children with cochlear implants. A total of 30 subjects (7 to 42 months) from Fatima Memorial College of Medicine and Dentistry were considered which were implanted with cochlea from October 2012 to December 2012. A pre-designed questionnaire was used for the data collection in order to collect the views from the parents of children with cochlear implants regarding the intelligibility of conversational speech produced by their children. It was noticed a 26 (86.7%) of the 30 youngsters who had cochlear implants were able to understand what was being said by their speakers. Moreover, parents and other listeners try to understand the conversation that cochlear implant's recipients make. It was concluded cochlear implants found useful and recommended for improving hearing of impaired individuals.*

Keyword: *Verbal communication, speech, intelligibility, Cochlear implant*

INTRODUCTION

Communication is only means by which one person can share thoughts and feelings with others (Carlson, 2020). This mechanism is present in all people by nature (Rakhimova et al., 2022). All communication is a way to convey information from one person to another (Naples and Ruckenstein, 2020). An individual's everyday communication status is only be estimated by their speech intelligibility (Rakhimova et al., 2022). Variables like gender, nonverbal intelligence, communication style, educational context, and extensive

technology use are all related to speech intelligibility (Varadarajan et al., 2021). The issue of evaluating speech comprehensibility needs study despite the numerous difficulties outlined above because decreased intelligibility is a major concern for many population of speech impaired people. However, after cochlear implantation, this comprehension has significantly improved in audibly impaired youngsters (Snels et al., 2019; Sharma et al., 2020).

Deafness is a word which is usually used for little or no hearing its degree can range from mild to severe or profound (Dornhoffer et al., 2020). World Health Organization (WHO) in 1980 described the word "deaf" for those whose hearing loss is so profound that they cannot benefit from any kind of amplification (hearing aid fitting) (Sharma et al., 2020). WHO (1980) established a classification such as mild (26-40 dB), moderate (41-55 dB), moderately severe (56-70 dB), severe, (71-91 dB), profound (>91db) based on a pure tone audiogram. While, an average of the hearing thresholds is 500, 1000, and 2000 Hz (McRackan et al., 2019).

Children who are very deaf (>90 dB loss) or totally deaf do not learn to speak and are frequently referred to as deaf mute or deaf and dumb (Teagle et al., 2019). The biggest flaw is hearing loss and speech was not developed in them since they had never heard speech (Gagnon et al., 2020). Therefore, nowadays an intervene in the form cochlear implant is available for these people to develop speech and to make them as an important member of society (Buchman et al., 2020; Dazert et al., 2020). The members started to use visual, aural, or tactile senses to express themselves (Deep et al., 2019). So, it is noticed that children who receive Cochlear Implants before the age of 5 demonstrate higher growth in their speech production skills than children who receive cochlear implants after this age (Zeitler et al., 2019). However, various other factors like gender, non-verbal intelligence, communication style, educational background, and extensive technology use also

influenced the speech capabilities (Messersmith et al., 2019). The development of speech in Cochlear Implants' recipients substantially varies among different people (Hoff et al., 2019). Longevity of deafness, age at onset, age of implantation, duration of Cochlear Implants, physiological or device factors such as the number of surviving spiral ganglion cells, electrode placement, insertion depth, electrical dynamic range, signal processing techniques, as well as additional psychological, educational, and social factors all are factors that affect an individual's variability (Plontke et al., 2020). Despite a variety of probable causes for the high inter-subject variability in speech intelligibility, using Cochlear Implants devices can assist to improve speech intelligibility (Galvin III et al., 2019).

The present study was planned to see the intelligibility of the children with cochlear implant. Moreover, role of speech session and various other parameters are also assessed via filling a questionnaire.

MATERIAL AND METHODS

Study Site

A total of 30 subjects from age of (7 to 42 months) with cochlear implants were considered for this study. The study was carried out with the children of Fatima Memorial College of Medicine and Dentistry.

Study Period

The study was conducted from October 2022 to December 2022.

Pre-Consent

A pre-consent was taken from parents/ participants of all the selected subjects.

Collection of Data

A pre-designed questionnaire was used to collect information about the intelligibility of conversational speech from the parents of cochlear implanted children. The questionnaire was consist of eight major questions further divided into various sub sections (Table 1). The obtained data was used to determine the overall intelligibility scores of conversational speech that produced by children with cochlear implants.

RESULTS

It was noticed in the present study a total of 30 subjects were selected and 12 were female and 18 were male. All the subjects were between seven months to forty two months old and with bilateral hear loss. The used questionnaire was consist of eight major questions which was further divided into various sub sections (Table 1). It was noticed in the study that in answer to first question four (13.3%) participants reported acquired hear loss, while 26 (86.7%) participants had congenital hear loss. While, in order to time of diagnosis 27 (90%) participants told that the child was diagnosed between the ages of 1 and 12 months, while only 3 (10%) participants reported their child was diagnosed after 36 months. Upon answer to speech therapy the subjects gave four different answers: one (3.3%) stated that it started at four months after cochlear implantation, and other (3.3) reported six months after cochlear implantation, while, 19 (63.3%) reported it began two months after cochlear implantation, and nine (30%) reported it began three months after cochlear implantation (Table 1). When asked how many speech therapy sessions had followed for cochlear implantation, 21 (69.9%) participants reported they had more than 4 speech therapy session per month. A 03 respondents (10%) said they had only one speech therapy session per month. While 06 respondents (20%) said they had only four sessions per month. In answer to question about subject's communication after cochlear implantation, 30 (100%) respondents reported that their child communicated vocally; none of them mentioned their child's use of sign language or gesticulation. The participants reply about intelligibility of their child’s speech was also asked, and 26 (86.7.0%) reported that their child speech was intelligible for them and only 4 (13.3%) participants had reported that their child speech was non-intelligible for them (Table 1).

Table 1. A predesigned questionnaire and its response

Questions	Response	%Frequency
Is Hearing Loss (HL) of child Congenital or Acquired?	Yes	26 (86.7%)
	No	4(13.3%)
Is HL of patients Unilateral or Bilateral?	Yes	30(100.0%)
	No	0
When the HL was first diagnosed?	1-12	20 (66. 6%)
	13 -24	2 (6. 6%)
	25- 36	5 (16. 6%)
	after 36	3 (10.0%)
What was age at the time of cochlear implant?	1-12	2(6.7%)
	13 -24	2(6.7%)
	25- 36	6(20.0%)
	after 36	20(66.6%)
When was speech therapy started after Cochlear Implant?	After 1 month	0
	After 2 months	19(63.3%)
	After 3 months	9(30%)
	After 4 months	1(3.3%)
	After 5 months	0
	After 6 months	1(3.3%)
No. of speech therapy sessions after Cochlear Implant?	1 per month	3(10%)
	2 per month	0
	3 per month	0
	4 per month	6(20%)
	More than 4 per month	21(69.9%)
How does your child communicate after cochlear implantation?	Verbal communication	30(100%)
	Non Verbal communication	0

If verbal then conversational speech is intelligible for you and others?	Yes	26(86.7 %)
	No	4 (13.3%)

DISCUSSION

It was noticed in the current study that most of the problem in considered subjects were congenital (86.7%) and bilateral (100%) and also be reported in various literature studies (Maes et al., 2014; Rine et al., 2013; Kimura et al., 2018). The most probable reasons for congenital diseases are cousin marriages, pressure from the structure of the family, and genetic alterations are a few potential causes of inheriting deafness. The outcomes clearly showed that 86.7% of children with cochlear implants could be understood verbally by an untrained listener (Katongo, 2015; Rogers, 2012). The findings showed that children with cochlear implants had ability to understood conversational speech and also reported by Geers et al. (2003). A question to participants was asked about how they learned that their child was deaf and different response were noticed with 66.6% reply after 36 months and same question was also asked by (Sodiqovna et al., 2020), three (10%) respondents stated they discovered their

child's handicap after the age of 36 months, whereas 27 (90%) indicated they made the discovery when the child was younger than 12 months. According to the results, the majority of respondents discovered their child's deafness while they were under 12 months old (Deep et al., 2021). It demonstrates that the signs of deafness were extremely obvious. The outcomes also indicated that parents were more concerned about their kids. It is significant to note that, despite implanting their child to regain auditory skills, the majority of parents were discovered to be more concerned with their children's verbal communication (Tarabichi et al., 2021). These findings, however, showed that 20 (66.7%) infants had their implants placed after the age of 36 months from the time of diagnosis, which is relevant given that early implantation is more important for the development of verbal

communication (Homans and Vroegop, 2021). Possible explanations for the delay in cochlear implantation include financial circumstances. The children with cochlear implants have speech therapy sessions and results revealed that the majority of parents began speech therapy for their children three months after cochlear implants, highlighting their concerns regarding verbal communication. It is important to keep in mind that rigorous speech treatment is more important for the development of verbal communication than interpreting conversational discourse (Clyne and Clyne, 1996). The results showed that 23 children (76.7%) reported receiving more than 4 speech therapy sessions per month, while 4 children (13.3%) reported receiving just 1 session per month and 3 children (10%) reported receiving only 4 sessions per month. Speech session bring more improvement as reported by Fuller et al. (2018). The participants were verbally communicating despite a delay in the cochlear implantation of young patients also reported by Binos et al. (2021). Only 4 (13.3%) respondents said their child was interacting with them using sign language, whereas nearly 26 (86.7%) said their child was verbally conversing. Those who were verbally communicating had speech that was understandable to both parents and strangers.

CONCLUSION

It was concluded in the study that cochlear implant was a way to treat congenital deafness. Moreover, conversational speech produced by the children with cochlear implant was intelligible for their parents and a significant progress was observed in it with an increased number of speech sessions. So, it is suggested by the more speech therapy sessions and proper care by the therapist and parents help to improve the intelligibility of the child and they can move towards normal quality of life.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

1. Binos P, Nirgianaki E, Psillas G (2021). How effective is auditory–verbal therapy (AVT) for building language development of children with cochlear implants? A systematic review. Life. 11(3): 239.
2. Buchman CA, Gifford RH, Haynes DS, Lenarz T, O'Donoghue G, Adunka O, Biever A, Briggs RJ, Carlson ML, Dai P (2020). Unilateral cochlear implants for severe, profound, or moderate sloping to profound bilateral sensorineural hearing loss: a systematic review and consensus statements. JAMA Otolaryngol. Head and Neck Surg. 146(10): 942-953.
3. Carlson ML (2020). Cochlear implantation in adults. New Engl. J. Med. 382(16): 1531-1542.
4. Clyne M, Clyne MG (1996). Inter-cultural communication at work: Cultural values in discourse. Cambridge University Press.
5. Dazert S, Thomas JP, Loth A, Zahnert T, Stöver T (2020). Cochlear Implantation: Diagnosis, Indications, and Auditory Rehabilitation Results. Deut. Ärzteblatt Int. 117(41): 690.
6. Deep NL, Dowling EM, Jethanamest D, Carlson ML (2019). Cochlear implantation: an overview. J. Neurol. Surg. Part B: Skull Base. 80(02): 169-177.
7. Deep NL, Gordon SA, Shapiro WH, Waltzman SB, Roland Jr J T, Friedmann DR (2021). Cochlear implantation in children with single-sided deafness. The Laryngoscope. 131(1): E271-E277.
8. Dornhoffer JR, Holcomb MA, Meyer TA, Dubno JR, McRackan TR (2020). Factors influencing time to cochlear implantation. Otology & neurotology: official publication of the American Otological Society, American Neurotology Society and

European Academy of Otology and Neurotol. 41(2): 173.

9. Fuller CD, Galvin III JJ, Maat B, Başkent D, Free RH (2018). Comparison of two music training approaches on music and speech perception in cochlear implant users. Trends Hear. 22.
10. Gagnon EB, Eskridge H, Brown KD (2020). Pediatric cochlear implant wear time and early language development. Cochlear Implan. int. 21(2): 92-97.
11. Galvin III, JJ, Fu QJ, Wilkinson, EP, Mills D, Hagan SC, Lupo J E, Padilla M, Shannon RV (2019). Benefits of cochlear implantation for single-sided deafness: data from the House Clinic-University of Southern California-University of California, Los Angeles Clinical Trial. Ear and Hearing. 40(4): 766-781.
12. Geers AE, Nicholas JG, Sedey AL (2003). Language skills of children with early cochlear implantation. Ear and hear. 24(1): 46S-58S.
13. Hoff S, Ryan M, Thomas D, Tournis E, Kenny H, Hajduk J, Young NM (2019). Safety and effectiveness of cochlear implantation of young children, including those with complicating conditions. Otol. Neurotol. 40(4): 454.
14. Homans NC, Vroegop JL (2021). Impact of face masks in public spaces during COVID-19 pandemic on daily life communication of cochlear implant users. Laryngos. Investig. Otolaryngol. 6(3): 531-539.
15. Katongo ME (2015). The role of music in selected speech intelligibility of learners with post lingual hearing impairment in selected special units in Lusaka District (Doctoral dissertation).
16. Kimura Y, Masuda T, Kaga K (2018). Vestibular function and gross motor development in 195 children with congenital hearing loss—assessment of inner ear malformations. Otol. Neurotol. 39(2): 196-205.
17. Maes L, De Kegel A, Van Waelvelde H, Dhooge I (2014). Association between vestibular function and motor performance in

hearing-impaired children. Otol. Neurotol. 35(10): e343-e347.

18. McRackan TR, Hand BN, Consortium CIQLD, Velozo C A, Dubno JR (2019). Cochlear implant quality of life (CIQOL): development of a profile instrument (CIQOL-35 Profile) and a global measure (CIQOL-10 Global). J. Speech Lang. Hear. Res. 62(9): 3554-3563.
19. Messersmith JJ, Entwisle L, Warren S, Scott M (2019). Clinical practice guidelines: Cochlear implants. J. Am. Acad. Audiol. 30(10): 827-844.
20. Naples JG, Ruckenstein MJ (2020). Cochlear implant. Otolaryngologic Clin. North Am. 53(1): 87-102.
21. Plontke SK, Fröhlich L, Wagner L, Kösling S, Götze G, Siebolts U, Liebau A, Rahne T (2020). How much cochlea do you need for cochlear implantation? Otol. Neurotol. 41(5): 694-703.
22. Rine RM, Wiener-Vacher S (2013). Evaluation and treatment of vestibular dysfunction in children. Neuro.Rehabilit. 32(3): 507-518.
23. Rogers E 2012. Thesis. Development and Evaluation Of The New Zealand. CHILDREN'S-BUILD-A-SENTENCE TEST (NZ Ch-BAS)
24. Sadikovna RK (2022). Hearing-speech rehabilitation of children with cochlear implants as a socio-pedagogical problem. Asian J. Multidimensional Res. 11(11): 6-9.
25. Sadikovna RK (2022). Objectives and tasks of cochlear implantation. Web of Scientist: Int. Scientific Res. J. 3(4): 1250-1255.
26. Sharma SD, Cushing SL, Papsin BC, Gordon KA (2020). Hearing and speech benefits of cochlear implantation in children: A review of the literature. Int. J. Pediat. Otorhinolaryngol. 133: 109984.
27. Snels C, IntHout J, Mylanus E, Huinck W, Dhooge I (2019). Hearing preservation in cochlear implant surgery: a meta-analysis. Otol. Neurotol. 40(2): 145-153.
28. Sodiqovna RK, Zulfiya A (2020). Formation of Independence Motivation Based on Rehabilitation Work with Children with Cochlear

Implants. Int. J. Integrat. Edu. 3(10): 310-312.

29. Tarabichi O, Jensen M, Hansen MR (2021). Advances in hearing preservation in cochlear implant surgery. Curr. Opi. Otolaryngol. Head Neck Surg. 29(5): 385-390.
30. Teagle HF, Park LR, Brown K D, Zdanski C, Pillsbury HC (2019). Pediatric cochlear implantation: A quarter century in review. Coch. Impl. Int. 20(6): 288-298.
31. Varadarajan VV, Sydlowski SA, Li MM, Anne S, Adunka OF (2021). Evolving criteria for adult and pediatric cochlear implantation. 100(1): 31-37.
32. Zeitler DM, Sladen DP, DeJong MD, Torres JH, Dorman MF, Carlson ML (2019). Cochlear implantation for single-sided deafness in children and adolescents. Int. J. Ped. Otorhinolaryngol. 118(1): 128-133.

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In Silico Analysis of MARS1 Gene to Elucidate Low-Frequency Variants Associated with Interstitial Lung and Liver Diseases

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ABSTRACT: *Mutation in MARS1 gene is linked to the development of Interstitial lung and liver disease. The current study aimed in silico analysis to predict the most harmful missense and spliced variants of MARS1 that damage the functionality of Methionyl-tRNA synthetase 1 (MARS 1), catalyses the ligation of methionine to tRNA and is essential for protein biosynthesis. A total of 492 variants were retrieved from the gnomAD database and analysed by CADD, 308 missense variants with PHRED score ≥ 20 were further analysed by CAPICE, META-SNP and CONDEL.85 SNPs detected with deleterious impact on protein structure by screening nsSNPs. Moreover, in-silico stability analysis was done by different tools like DynaMut, DUET, i-Stable2.0 and YASARA. MARS1 protein structure obtained from RCSB PDB (PDB ID: 5GL7) and UCSF Chimera was used for its visualisation. NetSurf-2.0 obtained the analysis of protein functioning by position of residue in the structure. Our results showed that the structure of proteins was significantly deleterious and protein motif and function were changed, we proceeded to use the PROSITE database to forecast the post-translation modification sites and four significant nsSNPs with protein structure change effects. Splice analysis was conducted by SPiCE, Human Splice Finder. It concludes in silico analysis, genes can determine likely pathogenic variation for further in vitro experimental study.*

Keyword: *Interstitial, Methionyl-tRNA synthetase, loss of mutation, gnomAD, Mutation prediction*

INTRODUCTION

Interstitial lung and liver disease (ILLD) is an autosomal recessive disorder characterised by its lipoprotein growth within alveoli, leading to constricted

lung and respiratory failure caused by alterations in the methionyl-tRNA synthetase 1(*MARS1)* gene. *MARS1* is a considerable candidate gene for association with Interstitial Lung and Liver Disease (Rips et al., 2018).

MARS codes methionyl-tRNA synthetase, which belongs to the class 1 family of the aminoacyl-tRNA synthetase (ARSs); such enzyme plays a significant role in protein synthesis by charging tRNAs with their cognate amino acids (Lenz et al., 2020).

Analysis of pathogenic variants is crucial to detect deleterious mutations that are found in the human genome. The human genome consists of the intronic and exonic regions, but the pathogenicity ratio is higher in the coding region (Blackstone 2018). Mutations are mainly related to single nucleotide polymorphism (SNPs) at their coding region, which includes the alteration of an amino acid that emanates the deformity of the protein's function (Bao et al., 2020).

GnomAD is an alliance of investigators seeking to organise exome and genome data from a broader scale into a summary that can provide comprehensive information to the scientific community (Karczewski et al., 2020). These mutations are filtered in CADD, widely used to detect deleterious missense mutations, and can score SNVs. In addition, it works on machine learning between *de novo* variants and the variants that are arisen and become anchored in the human population (Kircher et al., 2014).

Several missense tools like Meta-SNP, CAPICE, and CONDEL are used. Meta-SNP is based on the value of the Reliability Index (RI). The RI value ranges from 0 to 1; the mutations with RI scores less than 0.5 were expected to be harmful, whereas those with RI scores more than 0.5 were projected to be tolerated (Kumar et al., 2018). CAPICE is a new machine-learning-based technique for prioritising pathogenic variants such as SNVs and short InDels (Li et al., 2020). CONDEL is a copy number variation software program (CNV). Its output is composed of five other predictive tools such as Log R Pfam E-value (Clifford et al., 2004), MAPP (Stone and Sidow 2005,

Binkley et al., 2010), Mutation Assessor (Massessor) (Reva et al., 2007), Polyphen2 (PPH2),7 (Adzhubei et al., 2010) and SIFT,13–15 (Ng and Henikoff 2001, Kumar et al., 2009). SIFT was 0.85, Logre was 0.51, MAPP SIFT was 0.85, Logre was 0.51, MAPP (Clifford et al., 2004) was 0.06, Polyphen2 was 0.28, and Massessor was 0.26. Although the intrinsic scores of the five prediction tools differ in form, they all indicate the likelihood that an amino acid change would be approved at a specific place in a protein sequence.

On pathogenic variants, stability tests are applied. The objective is to calculate the difference in free energy upon protein folding. Furthermore, change in Gibbs free energy evaluates the impact of missense mutations on protein's stability. For intendment, various bioinformatics tools such as DYNAMUT (Rodrigues et al., 2018), DUET (Pires et al., 2014) and i-Stable2.0 (Chen et al., 2013) are practised.

The Fold X algorithm, one of the strong determinants of protein stability used for stabilising and destabilising estimation, calculated free energy change upon mutation (Li et al., 2009). UCSF Chimera is used to visualising the retrieved 3D structures and the unwanted interactions due to mutated residues (Pettersen et al., 2004). Post-translational modification analysis increases the complexity of proteomes. PTM sites are involved in mutations, specifically at phosphorylation sites. PTM was confirmed by ScanPROSITE (a protein database) in the *MARS1* Proteins to discover motifs, domains and interactions with other proteins (Hulo et al., 2006).

Different conservation-based tools, such as Netsurf 2.0**,** predicted solvent accessibility (Klausen et al., 2019), secondary structure, structural disorder, and backbone dihedral angles (Petersen et al., 2009). Consurf outputs a score, with 9 being the most conserved amino acid and 1 representing the most varied amino acid. (Ashkenazy et al., 2016) were performed. PROTEIN PLUS was used for ligand binding analysis by observing the change in ligand interaction with protein. (Fährrolfes et al., 2017).

To validate variation in 5'/3' splice sites, two bioinformatic tools SPiCE (Leman et al., 2020) and Human Splice Finder (HSF) (Tang et al., 2016), were performed. Several articles were published related to the role of SNPs in the MARS1 gene in different diseases, but there is still a need for computational analysis.

The recent study was aimed to determine the functional and structural consequences of nsSNPs in the coding region of the *MARS1* gene that is crucial in disease susceptibility through the bioinformatics tool. The majority of the mutations produce an effect on protein stability. The bioinformatics tool also predicted post-translational modification sites on protein structure.

MATERIAL AND METHODS

Retrieval and selection of variants of the *MARS1* gene

The gnomAD v2.1, Variation Viewer and DbSNP database were utilised to assess human *MARS1* variants. The UniProt database has been used to derive protein sequence (PDB ID: P56192) and SNPs Information from the *MARS1* gene. For the retrieving of variants, we implemented gnomAD in our analysis. The schematic diagram is in the following Fig.1.

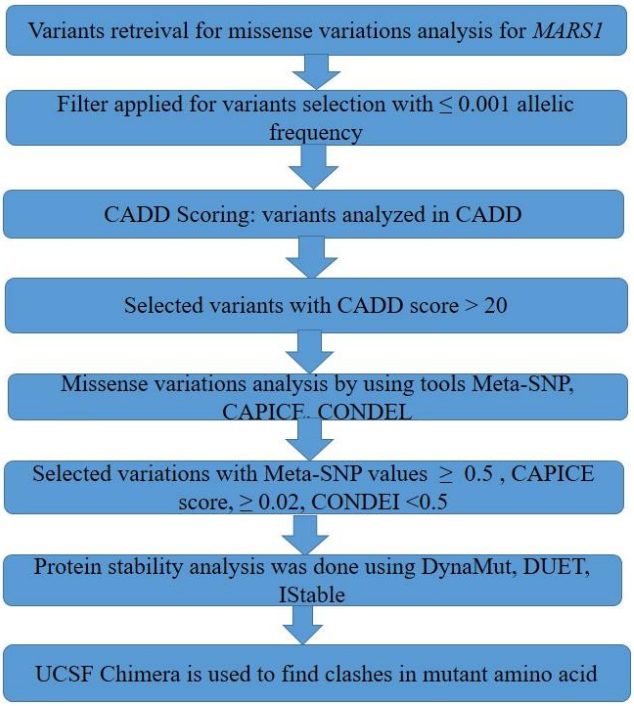

Fig.1 Streamline followed for missense variations analysis in *MARS1* gene

NON-SYNONYMOUS SINGLE NUCLEOTIDE POLYMORPHISM ANALYSIS

The following in silico tools are used to predict the effect of SNPs on protein function: Meta-SNP, CAPICE, and CONDEL.

Predicting the deleterious nsSNPs by Meta-SNP

Meta-SNP trained as a random forest-based binary classifier on the output capacity of four tools: SNAP, SIFT, PANTHER and PhD-SNP. As a meta classifier of the tool, its prediction is more accurate than the single tool. The predicted pathogenicity of a mutation depends upon the value of the Reliability Index (RI). The range of RI value is from 0 to 1. The pathogenicity score with an RI value < 0.5 was predicted as pathogenic, while the others with an RI value > 0.5 were predicted as tolerated.

Predicting the functional impact of deleterious nsSNPs by CAPICE

CAPICE is a supervised machine-learning-based model for prioritising pathogenic variants, including SNVs

and short InDels. It is trained on balanced data to annotate SNVs on 11 categorical features. Then, the retrieved variants are annotated accordingly to these features. Outputs are in the form of CAPICE scores predicting the pathogenicity of variations with a cutoff>0.02.

Predicting the functional impact of pathogenic nsSNPs by CONDEL

CONDEL is a software tool that predicts copy number variation (CNV)**.** The classifier is based on a combined predictive score of (Log R Pfam E-value (Logre), MAPP, Mutation Assessor (Massessor), Polyphen2 (PPH2), and SIFT. All retrieved variants were analysed with a combined classifier with output from Score 0 to 1; the higher score was characterised as deleterious.

PREDICTION OF PROTEIN STABILITY CHANGE

In silico analysis on protein stability aims to calculate the difference in free energy upon protein folding. The impact of missense mutations on protein's stability was evaluated by change in Gibbs free energy ($\Delta\Delta G$).

Predicting the protein stability changed by DynaMut, DUET and iStable 2.0

Missense mutation introduces a new amino acid which sometimes is incompatible with neighbouring residue, destabilising the protein and affecting its function. The retrieved variants were analysed with different stability prediction tools to predict the change in protein stability due to missense mutation. The DynaMut server predicts changes in free energy upon the folding and unfolding of the protein. Changes in free energy ($\Delta\Delta G$) of protein arise destabilised effect possessing threshold value >0.

PDB structure, wild type, mutant protein code and chain identification for visualising PDB structures and computing free-energy change ($\Delta\Delta G$) are needed to calculate the protein stability by DUET servers. The higher negative value will indicate a more impact of destabilisation.

iStable2.0 predicted protein stability depending on the vector-supported machine algorithm. Two input types,

structural or sequential protein information, can be supplied.

Prediction of energy minimisation and free energy change due to mutation by YASARA Fold X Program

YASARA software was downloaded along with plugin programs Fold X and Python by default installation to determine the effect of mutations on stability. The $\Delta\Delta G > 0$ value showed decreased stability while $\Delta\Delta G < 0$ value showed increased SNP stability.

PREDICTION OF PROTEIN STRUCTURAL PERSPECTIVE

Molecular Modeling by UCSF Chimera

For the confirmation of SNPs and 3D visualisation, we performed the analysis of the UCSF Chimera program. PDB ID fetched 3D structure (5GL7). The change in the amino acid at a specific position located in *MARS 1* chain A was generated by selecting an option of structure editing and then choosing rotamers. Native and mutant structures are visualised and downloaded as save PDB.

Clashes, Minimisation and labelling of molecule prediction

UCSF Chimera program found clashes that are unwanted interactions between native and mutant residue in Methionyl tRNA synthetase 1 protein. Energy minimisation is utilised to construct or refinish H- bond networks, eliminate unwanted contacts and lower total system energy in protein molecular modelling. A 3-letter amino acid code labelled residues with their perspective position.

Prediction of Post Translational Modification Sites on protein structure by UniProt and Prosite Scan

These are all the alterations following mRNA protein translation. These changes are important for the functioning of proteins or any other enzymes to be identified. The PTM enzymes detected a particular consensus sequence or motifs for these changes. In these specific locations, random mutation can also occur for change and cannot be noticed through activating enzymes, resulting in no modification of enzymes that cannot enable the protein

to work properly. We used ScanProsite to determine the PTM sites in the MARS1 Proteins to discover motifs, domains and interactions with other proteins in the FASTA or UniProt Sequence accession No. (P_56191) sequence. The results detailed changes in the location indicated.

ANALYSIS OF PROTEIN CONSERVATION

Prediction of Conserved residues by ConSurf

It is a method used in bioinformatics to compute the evolution of amino acid conservation by using an empirical Bayesian inference in the protein sequence. Assessment of the homologous sequences is dependent on evolutionary history. The importance of a residue can be observed through the conservational score: the more conserved residue, the more severe effect of its mutation on protein function. The degree of amino acid residue conservation with 50 homologous sequences was estimated. The method was chosen those significantly conserved residues for additional study at the high-risk nsSNPs sites. The conservation score is given together with a colour scheme. For example, the most conserved is score 9, while score 1 is the most varied amino acid.

Prediction of solvent accessibility prediction by NetSurf -2.0

The tool predicted secondary structure, structural disorder, backbone dihedral angles and solvent accessibility of amino acids to find the active site in the completely folded protein. Furthermore, characterising the position of residue in the protein, especially in the catalytic site, can be predicted by NetSurf -2.0. The mechanism for this prediction is based on Z, which can estimate the surfaces of proteins, but not their secondary structures. Its output consists of three subclasses, i.e., buried, partially buried and exposed protein regions in the protein.

Ligand Binding Analysis by Protein Plus

In some areas, the protein binds with a ligand known as a ligand binding site. These ligands are essential for allosteric conformation changes in the protein function. The mutation will impair

ligand binding to protein at these specific sites. To analyse the change in the interaction of the ligand with protein, we apply UCSF and PROTEIN PLUS. Proteins Plus focuses on interactions between proteins and ligands at the binding site. It may also detect protein pockets, generate ensembles or predict metal coordination.

EFFECT OF PREDICTED MUTATIONS ON SPLICING

Splicing proteins identify specific locations, but splicing doesn't happen to owe to undiscovered sites if a mutation replaces it. An estimation of spliceogenic variations that affect pre-mRNA splicing have been identified as the critical index of splicing in the silicon analyses, interrupting 5 and 3' splice sites or changing regulatory components 5 and 3.' Recent research has analysed the locations of regions that have predicted a prediction by using SPiCE, Human Splice Finder (HSF), for each variant to predict (5') donors and (3") accepters.

Prediction of Splice Sites by SPiCE

We used mRNA (NM_004990) transcript, chromosome position and reference position as input in the SPiCE server and modified amino acid variants. The resulting classifier calculates labels in protein sequences after uploading the FASTA file. Then, SPICE calculates the accepter and donor *MARS 1* gene site, and SPICE interpretation evaluates the probability score. The output is in the form of MES and SSF-Like scores that are calculated along with graphical representation. The SPiCE probability score range from 0-1; the higher value more will be the probability of disrupting the splice region.

Prediction of the impact of SNP located in splice site by HSF tool.

Human Splice Finder (HSF) identifies as well as forecasts mutations to increase or repress splicing patterns, identifying splicing locations for the acceptor and donor, and also the branch point and auxiliary sequences known for Exonic Splicing Enhancers (ESE) and Exonic Splicing Silencers (ESS).

NON-SYNONYMOUS SINGLE NUCLEOTIDE POLYMORPHISM ANALYSIS

The following in silico tools are used to predict the effect of SNPs on protein

function: Meta-SNP, CAPICE, and CONDEL.

Predicting the deleterious nsSNPs by Meta-SNP

Meta-SNP trained as a random forest-based binary classifier on the output capacity of four tools: SNAP, SIFT, PANTHER and PhD-SNP. As a meta classifier of the tool, its prediction is more accurate than the single tool. The predicted pathogenicity of a mutation depends upon the value of the Reliability Index (RI). The range of RI value is from 0 to 1. The pathogenicity score with an RI value < 0.5 was predicted as pathogenic, while the others with an RI value > 0.5 were predicted as tolerated.

Predicting the functional impact of deleterious nsSNPs by CAPICE

CAPICE is a supervised machine-learning-based model for prioritising pathogenic variants, including SNVs and short InDels. It is trained on balanced data to annotate SNVs on 11 categorical features. Then, the retrieved variants are annotated accordingly to these features. Outputs are in the form of CAPICE scores predicting the pathogenicity of variations with a cutoff>0.02. **Predicting the functional impact of pathogenic nsSNPs by CONDEL**

CONDEL is a software tool that predicts copy number variation (CNV)**.** The classifier is based on a combined predictive score of (Log R Pfam E-value (Logre), MAPP, Mutation Assessor (Massessor), Polyphen2 (PPH2), and SIFT. All retrieved variants were analysed with a combined classifier with output from Score 0 to 1; the higher score was characterised as deleterious.

PREDICTION OF PROTEIN STABILITY CHANGE

In silico analysis on protein stability aims to calculate the difference in free energy upon protein folding. The impact of missense mutations on protein's stability was evaluated by change in Gibbs free energy ($\Delta\Delta G$).

Predicting the protein stability changed by DynaMut, DUET and iStable 2.0

Missense mutation introduces a new amino acid which sometimes is incompatible with neighbouring residue, destabilising the protein and affecting its

function. The retrieved variants were analysed with different stability prediction tools to predict the change in protein stability due to missense mutation. The DynaMut server predicts changes in free energy upon the folding and unfolding of the protein. Changes in free energy ($\Delta\Delta G$) of protein arise destabilised effect possessing threshold value >0.

PDB structure, wild type, mutant protein code and chain identification for visualising PDB structures and computing free-energy change $(\Delta\Delta G)$ are needed to calculate the protein stability by DUET servers. The higher negative value will indicate a more impact of destabilisation.

iStable2.0 predicted protein stability depending on the vector-supported machine algorithm. Two input types, structural or sequential protein information, can be supplied.

Prediction of energy minimisation and free energy change due to mutation by YASARA Fold X Program

YASARA software was downloaded along with plugin programs Fold X and Python by default installation to determine the effect of mutations on stability. The $\Delta\Delta G > 0$ value showed decreased stability while $\Delta\Delta G < 0$ value showed increased SNP stability.

PREDICTION OF PROTEIN STRUCTURAL PERSPECTIVE

Molecular Modeling by UCSF Chimera

For the confirmation of SNPs and 3D visualisation, we performed the analysis of the UCSF Chimera program. PDB ID fetched 3D structure (5GL7). The change in the amino acid at a specific position located in *MARS 1* chain A was generated by selecting an option of structure editing and then choosing rotamers. Native and mutant structures are visualised and downloaded as save PDB.

Clashes, Minimisation and labelling of molecule prediction

UCSF Chimera program found clashes that are unwanted interactions between native and mutant residue in Methionyl tRNA synthetase 1 protein. Energy minimisation is utilised to construct or refinish H- bond networks, eliminate unwanted contacts and lower total

system energy in protein molecular modelling. A 3-letter amino acid code labelled residues with their perspective position.

Prediction of Post Translational Modification Sites on protein structure by UniProt and Prosite Scan

These are all the alterations following mRNA protein translation. These changes are important for the functioning of proteins or any other enzymes to be identified. The PTM enzymes detected a particular consensus sequence or motifs for these changes. In these specific locations, random mutation can also occur for change and cannot be noticed through activating enzymes, resulting in no modification of enzymes that cannot enable the protein to work properly. We used ScanProsite to determine the PTM sites in the MARS1 Proteins to discover motifs, domains and interactions with other proteins in the FASTA or UniProt Sequence accession No. (P_56191) sequence. The results detailed changes in the location indicated.

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RESULTS AND DISCUSSION

In silico analysis of deleterious nsSNPs were analysed by missense tools to predict the most pathogenic nsSNPs involved in disease. The resulting missense nsSNPs were performed with stability analysis to determine the stability changes upon mutation.

Analysis of Missense Variants

Total numbers of 492 variants were retrieved from these databases combined. 492 variants after applying VEP annotation filter and selecting missense variants with allelic frequency ≤ 0.001 and allelic count < 50 were then analysed using CADD. 308 variants were left after applying the filter on variants with CADD scoring ≥ 20. CAPICE, CONDEL and Meta-SNP were applied to measure the effect of single nucleotide polymorphism on pathogenicity and figure out the SNPs associated with the disease. Meta-SNP predicted 85 nsSNPs to be pathogenic with a threshold score of> 0.5. Our CAPICE analysis showed that 85 nsSNPs were identified with deleterious effects with a threshold score > 0.02.

Whereas CONDEL detected 85 nsSNPs as a pathogenic effect with a cutoff score > 0.5 on *MARS1* gene.

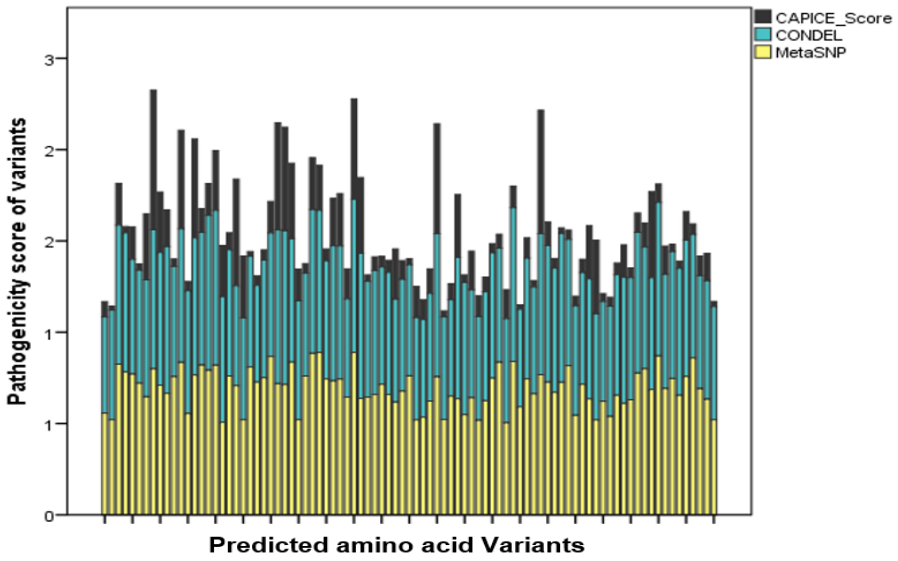

Fig.2 Graphical Representation of pathogenic missense variants in *MARS1*

After applying a combined cutoff filter of all missense analyses, we get 85 pathogenic variants. A study in 2019 was conducted in which Superoxide dismutase 3 (SOD3) were analysed through *in silico* analysis. The two mutations p.A91T and p.R231G were discovered to be deleterious for ligand binding analysis as a result of molecular dynamic simulation (Pereira et al., 2019).

Stability Analysis of Protein

Filter 85 missense variants were then analysed for stability change using protein stability predicting tools; DynaMut, DUET and iStable. The combined outcome of these tools demonstrates that 38 variants destabilised the protein structure, as shown in Fig. 3.

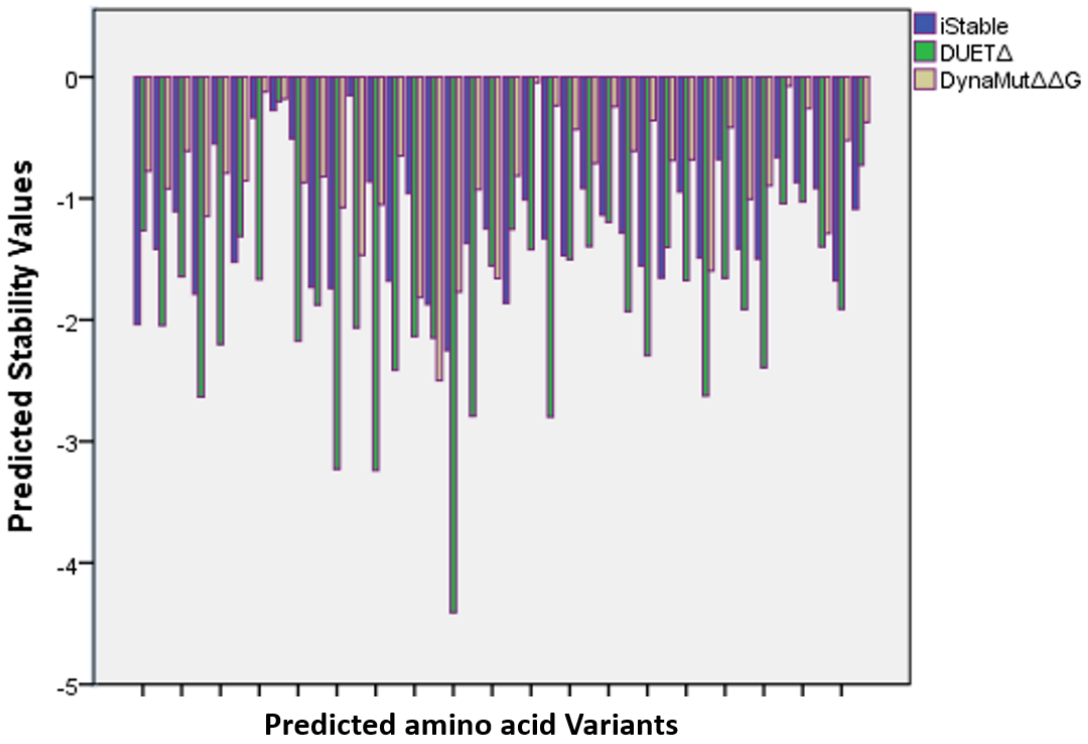

Fig. 3. Distribution of stabilising and destabilising nsSNPs upon stability change

Clashes Findings by UCSF Chimera

To visualise amino-acid mutations that disrupt the wild-type interaction with other residues, UCSF Chimera was used. Clashes which are unwanted interactions were identified when native amino acid was modified to a mutant amino acid for visualisation of this change in protein structure. Any change produced due to wild and mutant amino-acid residue may disturb the domain and have a loss of interactions that cause damage to protein structure. We analysed 85 filtered pathogenic variants, of which only 14 mutations showed clashes. Clashes between wild and mutant-type residues, as red-coloured lines between residues shown in Fig. 4. Results indicated that 14 mutations revealed clashes between wild and mutant type, i.e., *p.Ile285Phe*, *p.Gly310Trp*, *p.Pro329His*, *p.Cys408Trp*, *p.Ser484Pro*, *p.Phe551Leu*, *p.Tyr589His*, *p.Arg618His*,

p.Leu622Pro, *p.Arg625Trp*, *p.Leu681Pro*, *p.Ser712Phe*, *p.Asn716Lys*, *p.Gly796Val*.

Five mutations showed a higher no. of clashes (*p.Ile285Phe* (11 clashes), *p.Gly310Trp*(16 clashes), *p.Pro329His (*27 clashes), *p.Cys408Trp (*26 clashes), *p.Tyr589His* (26 clashes) have significantly affected the destabilisation of protein structure. Some mutations show a lesser number of clashes, such as *p.Ser484Pro* (7 clashes), *p.Phe551Leu* (4 clashes), *p.Arg618His* (8 clashes),), *p.Arg625Trp* (8 clashes), *p.Arg625Trp* (8 clashes) whereas the two remaining show only clash i.e.,.*p.Leu622Pro* (1 clash), and *p.Leu681Pro* (1 clash).

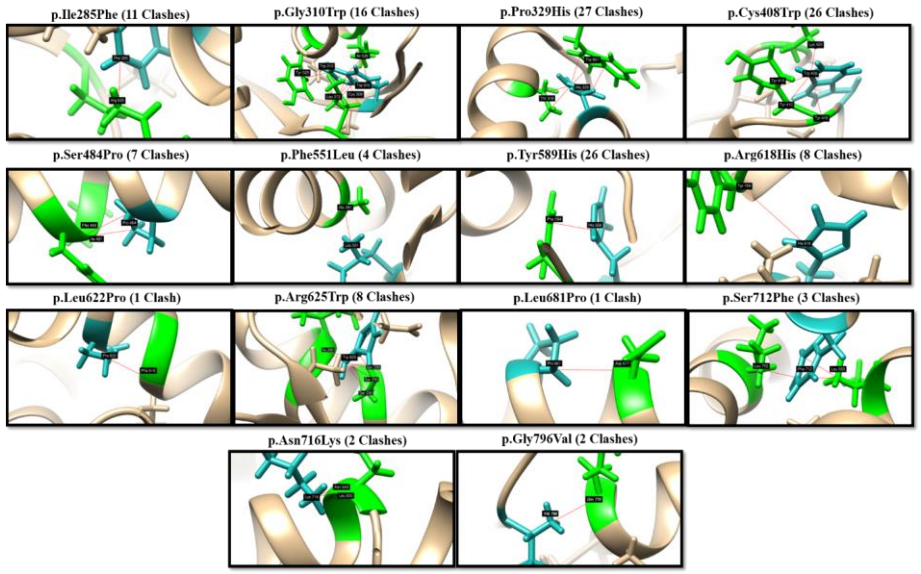

Fig. 4. Clashes finding due to mutations in *MARS 1* protein structure using chimera

Post Translational Modification (PTM) Analysis of *MARS1* Gene:

There have also been some mutations detected in protein Post-translational sites for modification. The 3 PTMs were found to be phosphorylation sites with the various types of kinases with their unique circumstances for our reported amino-acid substitution positions. The use of PROSITE (protein database) PTM (phosphorylation sites involving several kinases) has been confirmed, and these sites are monitored and visualised with UCSF Chimera protein structure. Fig.5a shows changes to the protein domain of methionyl-tRNA synthetase1 that involved UniProt predicted N-linked Glycosylation at p.Tyr532Cys. *Tyr532Cys* (ScanProsite site) showing (yellow) protein in fig.5a. As mutation stated, *p.Tyr532Cys* (a), Acidic Tyrosine, if mutated into Cysteine, is an uncharged amino acid and thus modifies the biochemical properties of nearby residues and may initiate the creation of a new N-linked Glycosylation site due to cysteine presence. In Fig. 5(b), ScanProsite predicted two phosphotheronin-implied sites at *p.Gln330Pro* and *p.Thr328Ile* highlighted in protein at (green). *p.Gln330Pro*, as indicated by mutation. Polar non charged glutamine mutating into proline which is uncharged amino acid, thus changing the residue's biochemical properties.

Another phosphorylation site (phosphothreonine) was observed at position 328 that may be disrupted by *p.Thr328Ile* reported mutations. (Orange and blue coloured site of the phosphothreonine affected. Also, in *p.Arg414Trp* Fig. 5(c) ScanProsite predicted one site participating in the RSD (cell attachment sequence).

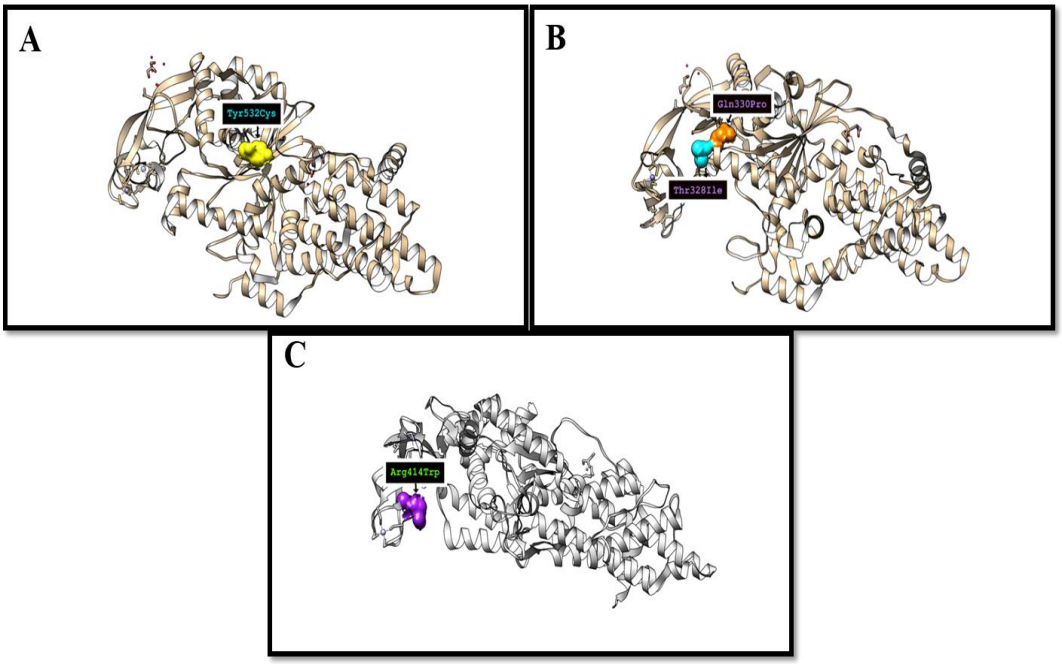

Fig. 5. Missense mutation highlighted in predicted PTMs sites

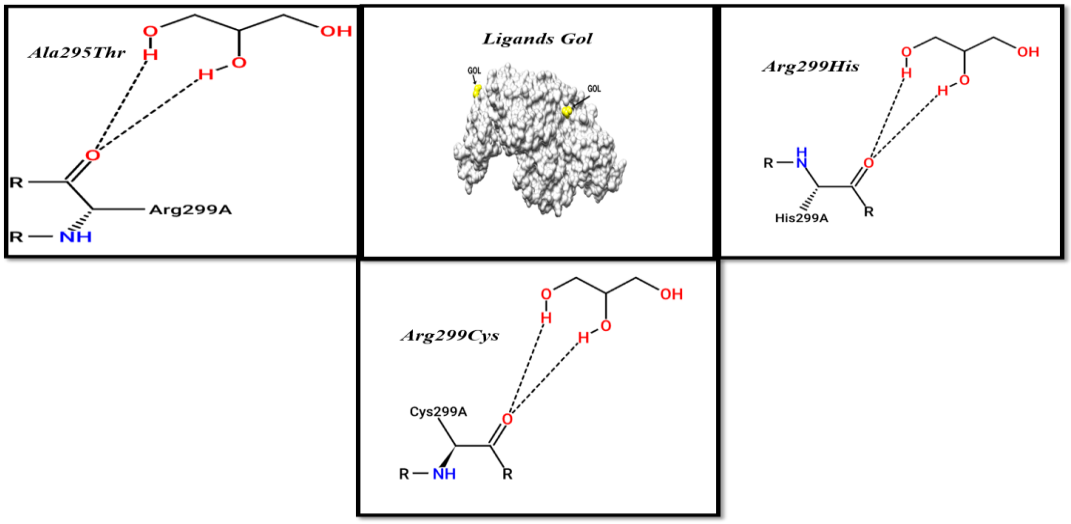

Fig. 6. The ligand highlighted with yellow (by using chimera), while the right end shows that residue **GOL_A_903** binding can interrupt the binding by mutation at *p.Arg299Cys, p.Arg299His, and p.Al295Thr* position

Protein Conservation and Secondary Structure Analysis of MARS1 Gene:

Predicted mutations at Ptm sites are then further validated by structural tools. The variant should be exposed and conserved for exposure to the modification enzyme. Consurf was used to anticipate our candidate protein structures for the conservation area or sequence. The more a protein is conserved, the more likely the protein is to mutate. In evolutionary research, conservation is vital. The slower the rate of conservation, the greater the protein structure will be conserved. The Consurf tool has been used to confirm whether the reported mutation is in the conservation area. NetSurf-2 anticipated secondary structures or to examine the buried or exposed amino acid on the surface.

Table.1 Protein conservation prediction using ConSurf and solvent accessibility, secondary structure prediction by NetSurfP-2.0

ConSurf			**NetSurfP-2.0**
Protein Change	**Score**	**Color**	**Sec. Structure/exposed/Buried**
p.Tyr532Cys	0.499	3	Exposed
p.Arg414Trp	-0.139	6	Exposed
p.Gln330Pro	-0.454	7	Exposed
p.Thr328Ile	-0.441	7	Exposed

Ligand Binding Analysis

The protein interacts with a ligand in certain places, known as a binding site. The mutation in these particular locations will disrupt ligand binding to protein. UCSF Chimera and PROTEIN PLUS are being used. The results showed that 3 variants at ligand binding sites could affect the binding of ligands to the protein. these variants p.Arg299His, p.Arg299Cys, and p.Ala295Thr as shown in the Fig.6.

Splice Site Variant analysis

10 variants were obtained from the gnomAD database to predict splice site defects.

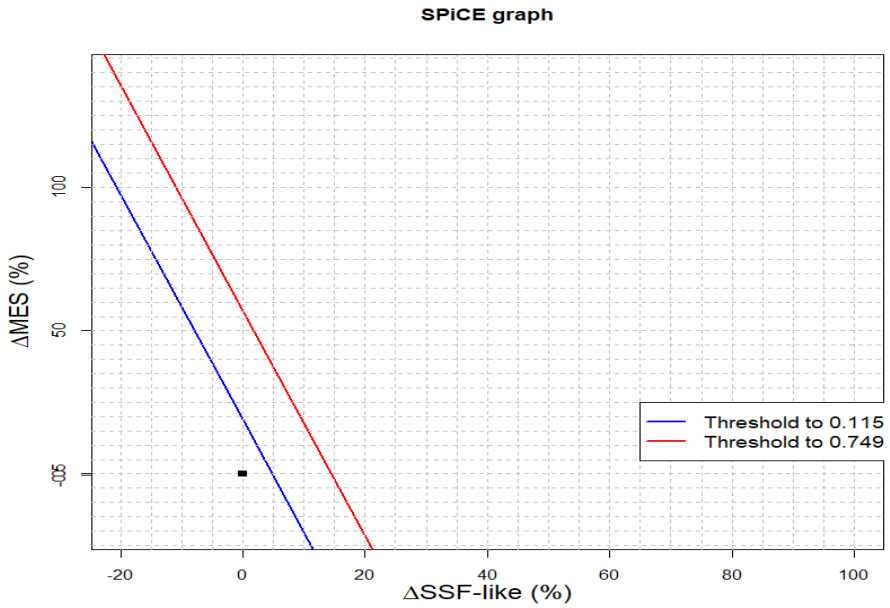

Fig.7 Graphical Representation of SPiCE Results

These variants have begun identifying the 5'/3' slice sites in the *MARS1* gene using several splicing tools. In 2014, a study was conducted to compare the missing rates of splice mutations which showed HSF with a minimum missing rate of 66 (Jian et al., 2014).

In this study utilising two bioinformatics methods to validate 5'/3' splice site (SPiCE and HSF). SPICE showed significant results in fig.7. However, HSF does not have a substantial *MARS1* gene interpretation suggesting that the splicing role of the *MARS1* gene is irrelevant.

The red colour schematic indicating the limit values and the points showing variations below the red scheme are the variants that are very unlikely or unlikely to alter the splicing mechanism. Our study can be a preliminary step for laboratory study. These identified variants can be the confident mutation of *MARS1* associated with Interstitial lung and liver disease. The structural analysis and functional analysis of these missense variants specify the effect of the variant on the protein.

CONCLUSION

Interstitial lung and liver disease is an autosomal recessive condition characterised by lipoprotein growth within the alveoli, leading to restrictive lung and respiratory failure. *MARS1* is a gene that codes for proteins. Interstitial Lung and Liver Disease associated with *MARS1*. An *in silico* study showed out of 492 retrieved variants 85 are significant highly pathogenic mutations with a valuable role in the function and structure of the *MARS1* gene. In addition, 38 missense pathogenic variants are responsible for disrupting the stability of protein structure. 14 variants have shown unwanted interactions with neighboring residues in the protein. Among highly pathogenic nsSNPs, only four nsSNPs predicted by PROSITE database to be in motif of Methionyl-tRNA Synthetase 1 protein and cause higher probability of interstitial lung and liver disease. *In silico* analysis in this study would help the researchers to understand the genetics of Interstitial lung and liver disorder and identify the mutations in *MARS1* gene that cause this disorder.

The reported SNPs analysis in *MARS1* gene can be further analysed by following wet lab experimental work or can be observed in animal models.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

1. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR (2010). A method and server for predicting damaging missense mutations. Nat. Methods. 7(4): 248-9.
2. Ashkenazy H, Abadi S, Martz E, Chay O, Mayrose I, Pupko T, Ben-Tal N (2016). ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. Nucl. Acids Res. 8: 44(W1):W344-50.
3. Bao S, Zhao H, Yuan J, Fan D, Zhang Z, Su J, Zhou M (2020). Computational identification of mutator-derived lncRNA signatures of genome instability for improving the clinical outcome of cancers: a case study in breast cancer. Brief. Bioinformat. 21(5): 1742-1755.
4. Binkley J, Karra K, Kirby A, Hosobuchi M, Stone EA, Sidow A (2010). ProPhylER: a curated online resource for protein function and structure based on evolutionary constraint analyses. Genome Res. 20(1): 142-154.
5. Blackstone C (2018). Hereditary spastic paraplegia. Handbook of clinical neurology 148: 633-652.
6. Chen CW, Lin J, Chu YW (2013). iStable: off-the-shelf predictor integration for predicting protein stability changes. Bio. Med. Cent. InBMC bioinformatics 14(2): 1-14.
7. Clifford RJ, Edmonson MN, Nguyen C, Buetow KH (2004). Large-scale analysis of non-synonymous coding region single nucleotide polymorphisms. Bioinformat. 20(7): 1006-1014.

8. Fährrolfes R, Bietz S, Flachsenberg F, Meyder A, Nittinger E, Otto T, Volkamer A, Rarey M (2017). Proteins Plus: a web portal for structure analysis of macromolecules. Nucl. Acids Res. 45(W1): W337-W343.
9. Hulo N, Bairoch A, Bulliard V, Cerutti L, De Castro E, Langendijk-Genevaux PS, Pagni M, Sigrist CJ (2006). The PROSITE database. Nucl. Acids Res. 34(1): D227-D230.
10. Jian X, Boerwinkle E, Liu X (2014). In silico prediction of splice-altering single nucleotide variants in the human genome. Nucl. Acids Res. 42(22): 13534-13544.
11. Karczewski, K. J., et al. (2020). "The mutational constraint spectrum quantified from variation in 141,456 humans." Nature 581(7809): 434-443.
12. Kircher M, Witten DM, Jain P, O'roak BJ, Cooper GM, Shendure J (2014). A general framework for estimating the relative pathogenicity of human genetic variants. Nat. Gen. 46(3): 310-315.
13. Klausen MS, Jespersen MC, Nielsen H, Jensen KK, Jurtz VI, Soenderby CK, Sommer MO, Winther O, Nielsen M, Petersen B, Marcatili P (2019). NetSurfP-2.0: Improved prediction of protein structural features by integrated deep learning. Prot. 87(6): 520-527.
14. Kumar DT, Emerald LJ, Doss CG, Sneha P, Siva R, Jebaraj WC, Zayed H (2018). Computational approach to unravel the impact of missense mutations of proteins (D2HGDH and IDH2) causing D-2-hydroxyglutaric aciduria 2. Metabol. Brain Dis. 33(5): 1699-1710.
15. Kumar P, Henikoff S, Ng PC (2009). Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat. Protocol. 4(7): 1073-1081.
16. Leman R, Gaildrat P, Le Gac G, Ka C, Fichou Y, Audrezet MP, Caux-Moncoutier V, Caputo SM, Boutry-Kryza N, Léone M, Mazoyer S (2020). Novel diagnostic tool for prediction of variant spliceogenicity derived from a set of 395 combined

in silico/in vitro studies: an international collaborative effort. Nucl. Acids Res. 48(3): 1600-1601.

17. Lenz D, Stahl M, Seidl E, Schöndorf D, Brennenstuhl H, Gesenhues F, Heinzmann T, Longerich T, Mendes MI, Prokisch H, Salomons GS (2020). Rescue of respiratory failure in pulmonary alveolar proteinosis due to pathogenic MARS1 variants. Pediat. Pulmonol. 55(11): 3057-3066.
18. Li B, Krishnan VG, Mort ME, Xin F, Kamati KK, Cooper DN, Mooney SD, Radivojac P (2009). Automated inference of molecular mechanisms of disease from amino acid substitutions. Bioinformatics 25(21): 2744-2750.
19. Li S, van der Velde KJ, De Ridder D, Van Dijk AD, Soudis D, Zwerwer LR, Deelen P, Hendriksen D, Charbon B, Van Gijn ME, Abbott K (2020). CAPICE: a computational method for Consequence-Agnostic Pathogenicity Interpretation of Clinical Exome variations. Genome Med. 12(1): 1-11.
20. Ng PC, Henikoff S (2001). "Predicting deleterious amino acid substitutions." Genome research 11(5): 863-874.
21. Pereira GRC, Da Silva AN R, Do Nascimento SS, De Mesquita JF (2019). In silico analysis and molecular dynamics simulation of human superoxide dismutase 3 (SOD3) genetic variants. J. Cell. Biochem. 120(3): 3583-3598.
22. Petersen B, Petersen TN, Andersen P, Nielsen M, Lundegaard C (2009). A generic method for assignment of reliability scores applied to solvent accessibility predictions. BMC Struc. Biol. 9(1): 1-10.
23. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004). UCSF Chimera—a visualisation system for exploratory research and analysis. J. Comput. Chem. 25(13): 1605-1612.
24. Pires DE, Ascher DB, Blundell TL (2014). DUET: a server for predicting effects of mutations on protein stability using an integrated computational approach. Nucl. Acids Res. 42(W1): W314-W319.

25. Reva B, Antipin Y, Sander C (2007). Determinants of protein function revealed by combinatorial entropy optimisation. Gen. Biol. 8(11): 1-15.
26. Rips J, Meyer-Schuman R, Breuer O, Tsabari R, Shaag A, Revel-Vilk S, Reif S, Elpeleg O, Antonellis A, Harel T (2018). MARS variant associated with both recessive interstitial lung and liver disease and dominant Charcot-Marie-Tooth disease. Europ. J. Med. Gen. 61(10): 616-620.
27. Rodrigues CH, Pires DE, Ascher DB (2018). DynaMut: predicting the impact of mutations on protein conformation, flexibility and stability. Nucl. Acids Res. 46(W1): W350-W355.
28. Stone EA, Sidow A (2005). Physicochemical constraint violation by missense substitutions mediates impairment of protein function and disease severity. Gen. Res. 15(7): 978-986.
29. Tang R, Prosser DO, Love DR (2016). Evaluation of bioinformatic programmes for the analysis of variants within splice site consensus regions. Adv. Bioinform.

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Lipid Variations as Prognosticators of Cardiovascular Risks in Dairy Cattle with Mastitis

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ABSTRACT: *Clinical mastitis a deadly problem for dairy farmers and economy. Current study was designed to check serum lipid profile alteration with respect to cardiovascular diseases in clinical mastitis cattle. A total of sixty five samples were collected and divided into two groups for this case control study on the basis of clinical examination. For analysis, 40 cases and 25 controls were included. Statistical analysis was done by applying student "t" test using GraphPad Prism software 6.0. There was non-significant reduction (P=0.3) of serum total cholesterol (TC) and pronounced elevation (P=0.01) of triglyceride (TG) in cases as compared to controls. Mastitis group presented mild decrease (P=0.07) in high density lipoprotein-cholesterol (HDL-C) and highly significant elevation (P=0.0003) of low density lipoprotein (LDL-C) as compared to healthy cattle. Moreover, diseased cattle also manifested marked elevation (P=0.01) of very low density lipoprotein-cholesterol (VLDL-C) as compared to controls. Conclusively, clinical mastitis is associated with painful, swollen udder and abnormalities in milk. It also contributes to dyslipidaemia that could be used as useful indicator for evaluation of cardiovascular risks in cattle with clinical mastitis.*

Keyword: *Dairy cattle, clinical mastitis, cardiovascular disorders, dyslipidaemia*

INTRODUCTION

Mastitis is an infection of udder tissue that induces physical and bacterial changes in milk as well as pathological alterations in the mammary gland (Radostits et al., 2006; Ashraf and Imran, 2020). It is a multi-factorial disease that affects dairy cows on a global scale and has a significant economic impact (Das et al., 2018). Due to reduced milk production, poor milk quality, higher treatment costs and early culling of infected animals, it is becoming more important disorder

(Qayyum et al., 2016). The disease is classified as clinical or subclinical on the basis of severity of udder inflammation. Depending on the causative pathogen it may be environmental or contagious in origin (Belay et al., 2022).

Clinical mastitis is the destructive malady of dairy animals, which results in huge economic losses to the dairy industry of Pakistan (Hameed et al., 2012). It can be detected by physical examination of the animal. It is characterised by the increased body temperature, discoloration, swelling, pain and discomfort in the mammary gland. Physical and chemical alterations in milk were also observed in clinical findings (Sarvesha et al., 2017).

The prevalence of mastitis is less during first lactation stage while its occurrence become higher with increasing number of lactations. Its occurrence is also linked with exotic breeds that are more susceptible to mastitis (Sadashiv et al., 2014). The ineffectiveness of treatment for mastitis is related to a number of factors, including inadequate veterinary care, antibiotic resistance, pathological changes in udder tissue and numerous causative pathogens (Adesola, 2012).

Lipid metabolism is challenged in dairy cows significantly during periparturient period to meet the energy requirements (Turk et al., 2013). Alteration in serum lipid profile is the crucial component of the energy needs and physiology of the transition dairy cows (Gross et al., 2013). Alterations in lipid profile in mastitic cattle have been reported (Kovačić et al., 2019; Abdel-Hamied and Mahmoud, 2020). However, available literature about the effect of clinical mastitis on lipid profile in cattle is still scarce.

According to our knowledge, there is no published data on cardiovascular disease (CVDs) assessment in mastitic cattle in Pakistan. In order to evaluate risks of CVDs in dairy cattle suffering from clinical mastitis, quantitative variations of lipid profile are investigated in present study.

MATERIALS AND METHODS

Sampling Facility and study design

The study was approved by the Ethical Review Committee of Institute of Zoology, University of the Punjab, Lahore. The research was carried on a total of 65 cattle, 3 to 7 years old, in their first to fifth parity belonging to the rural farms of Pattoki, District: Kasur and Bhera, District: Sargodha in Punjab province of Pakistan.

Study was performed from December 2021 to February 2022. Dairy cattle were kept in rural setups. All animals were fed with mixed ration and milked by hand, twice a day. Before sampling, a comprehensive proforma was prepared to record the etiological factors i.e., parity number, lactation period, age, milking method, milk yield, farm condition and common feed.

Visual examination of udder and teats was done on farm. California mastitis test (CMT) was performed to distinguish between healthy and infected cattle. On basis CMT screening test, cattle were categorized in two groups i.e., Control group and Mastitis group. The control group was comprised of healthy cattle. While, mastitis group included cattle with clinical symptoms like udder infection, abnormal milk production, reduced appetite, ruminant contraction, elevated respiratory and heart rate, water loss and hyperthermia.

Blood samples were collected from jugular vein in aseptic conditions. After phlebotomy, samples were left for thirty minutes at room temperature and centrifuged for fifteen minutes at 3000 rpm to collect serum. Then, the serum was stored at -80°C, until further biochemical usage.

Serum levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) of control and mastitis group were evaluated using commercially available kits of "Monlab", Spain through chemistry analyzer (Robert Riele Photometer 5010). Whereas, levels of very low density lipoprotein cholesterol (VLDL-C) were calculated

by the method of Friedewald et al. (1972).

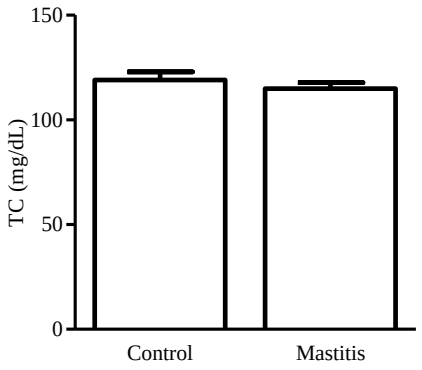
STATISTICAL ANALYSIS

Biochemical comparison between control and mastitis group was done by applying independent student "t" test using Graph Pad Prism version 6.0 software. Data of individual groups was expressed as Mean ± SEM. Mean values were significant at significance level $P \leq 0.05$.

RESULTS

Table 1 indicated the overall comparison of lipid profile in control and mastitis groups. Control vs mastitis group comparison demonstrated non-significant difference (P=0.3) with 3% decrease in TC levels in mastitis group as compared to controls (Figure 1a). The significant increase (P=0.01) of 13% in TG levels was found in mastitis group as compared to controls. (Figure 1b). While, non-significant difference (P=0.07) with 12% reduction of HDL-C was noticed in mastitis group, when compared with Controls (Figure 1c). Moreover, control vs mastitis group comparison depicted prominent difference (P=0.0003) with 49% elevation of LDL-C levels in mastitis group (Figure 1d). Lastly, statistically marked difference (P=0.01) was observed in mastitis group with 13% increase of VLDL-C as compared to controls (Figure 1e).

(a)



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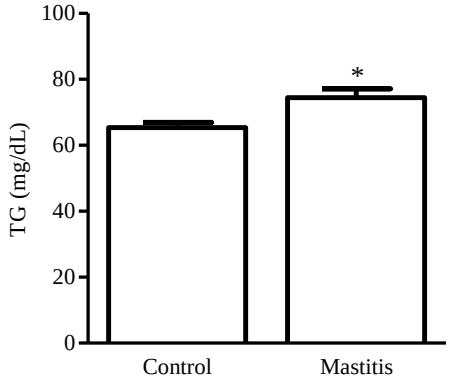

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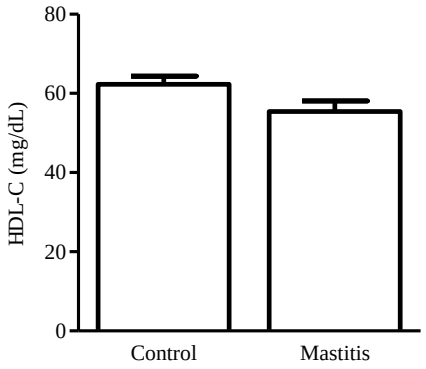

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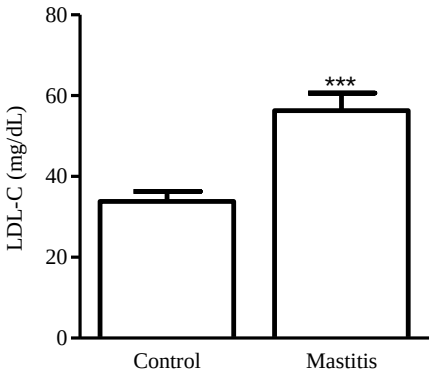

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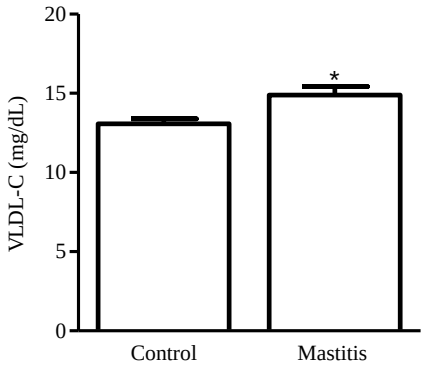

Figure 1: (a-e): An overall presentation of serum total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C). Values

are Mean ± SEM in control and mastitis group. Significant at *P ≤ 0.05 and ***P ≤ 0.001

Table 1: A comprehensive presentation of lipid profile in control and mastitis cattle

Parameters	**Mean ± SEM**		**P-value**	**% Difference**
	Control (n=25)	**Mastitis** (n=40)		
TC (mg/dL)	119.00± 3.88	115±2.83	0.30	3↓
TG (mg/dL)	65.36±1.61	74.45±2.65	0.01	13↑*
HDL-C (mg/dL)	62.28±2.08	55.45±2.64	0.07	12↓
LDL-C (mg/dL)	33.81±2.58	56.29±4.37	0.0003	49↑***
VLDL-C(mg/dL)	13.07±0.32	14.89±0.53	0.01	13↑*

↓: Decrease, **↑:** Increase.

, *** indicate significance at P ≤ 0.05, and 0.001, respectively

TC; Total cholesterol, TG; Triglyceride, HDL-C; High density lipoprotein cholesterol, LDL-C; Low density lipoprotein-cholesterol and VLD-L; Very low-density lipoprotein-cholesterol.

DISCUSSION

Present study determines changes in lipid biochemical parameters of cattle suffering from clinical mastitis and their association with cardiovascular risks. Mastitis is an inflammatory response due to pathogen invasion in the mammary gland. Macrophages, leukocytes and other inflammatory cells create reactive oxygen species (ROS) during an inflammatory reaction, which helps to kill bacteria but also damages tissues around them (Pham, 2006). Oxidative stress may develop due to ROS. High amount of ROS and lipid hydroperoxides produced from oxidative stress may also contribute to the death of cells and tissues (Ryman et al., 2015). Moreover, organoleptic properties of milk are degraded by excessive ROS accumulation in milk and lowers milk quality (Novac et al., 2022).

In the current study, milk presented physical abnormalities like blood flakes, abnormal consistency, pus and discolouration. Poor quality milk was not recorded in healthy cattle. Similar trends were observed by Radostits et al. (2006).

Changes in lipid metabolism occur during acute phase response (APR) including alterations in the HDL particle. Antioxidant proteins are removed from HDL-C particle during inflammatory response, whereas, proinflammatory proteins enriched it (Feingold and Grunfeld, 2010). Alteration in serum lipid profile and lipid metabolism is important for the energy requirements and affects the physiology of peripartum or transition cows (Gross et al., 2013; Arfuso et al., 2016) but also during high lactation periods and later during some pathological diseases i.e., subclinical and clinical mastitis (Kovačić et al., 2019).

Cholesterol serves as a building block for all steroid hormones, bile acids and is essential for transmembrane signalling, membrane trafficking, and cell growth (Fernández et al., 2004). Inspite its significance, imbalance in cholesterol amounts may have detrimental effects on cells and can result in conditions like atherosclerosis (Maxfield and Tabas, 2005). Our findings demonstrate non-significant reduction in serum total cholesterol (TC) levels in cattle with clinical mastitis when compared to healthy controls. Ali et al. (2017) recorded similar results in plasma samples of cows affected with clinical mastitis. It was reported by Kovačić et al. (2019) that TC decreases during inflammation. Moreover, inflammatory mediators such as LPS, TNF and IL-2 lower blood cholesterol concentrations (Khovidhunkit et al., 2004).

Triglycerides (TG) are major source of energy, produced by fatty acids and glycerol combination (Walker et al., 1990). Formation of TG occur in liver and it is packaged in form of very low density lipoprotein (VLDL). Clearance of triglyceride rich lipoproteins (TGRLs) is catalysed by lipoprotein lipase (LPL) and ineffective

delipidation of TGRLs induce production of cholesterol enriched remnants. Small sized remnants pass through endothelial layer and taken up by arterial wall's macrophages (Borén and Williams, 2016). These remnants and cytotoxic free fatty acids can give rise to proatherogenic adhesion molecules and inflammatory mediators (Nordestgaard, 2016; Sandesara et al., 2019).

Present study has shown significantly higher concentrations of serum TG in cattle with clinical mastitis than in healthy cattle. These results are in accordance with (Kovačić et al., 2019). Elevated levels of triglycerides have been associated with increased production of VLDL due to insulin resistance, which results in formation of small dense LDL molecules, triglyceride rich atherogenic remnants and HDL particles (Adiels et al., 2008).

Metabolic dysregulation results in hypertriglyceridemia due to high production of VLDL, delayed clearance of remnants by liver and disturbed activity of lipoprotein lipase in peripheral tissues. As far as humans are concerned, higher levels of TG and TGRL remnants have been documented as risk factors for the development of CVDs (Hassing et al., 2012). Hence, it can be depicted that elevated TG concentrations can increase chances of CVDs in animals.

Normal levels of HDL-C not only play important role as antioxidative, anti-apoptotic and anti-inflammatory particle but also prevents the risks of atherosclerosis (Ali et al., 2012). In our study, HDL-C concentrations in mastitis group were slightly lower than in healthy controls. It is most likely as a result of lipoprotein particle remodelling and cholesterol translocation from HDL to other lipoprotein particles (Tabet and Rye, 2009). According to Feingold and Grunfeld (2010), cholesterol reverse transport is decreased during APR. Moreover, reduced serum HDL-C levels may be due to impaired liver secretion of apolipoprotein A, which plays primary role in formation of HDL-C (Esteve et al., 2005). Hence, it

increases chances of atherosclerotic events.

LDL-C particles are the important carriers of cholesterol in bloodstream (Trinick and Duly, 2005). LDL particles in circulation are taken up by endothelial lining of arterial wall and then trapped in arterial intima. They may undergo oxidation and ingested by macrophages to promote atherogenesis (Freeman, 2010). LDL-C is investigated as the most important atherogenic lipoprotein (Hirayama and Miida, 2012). During inflammatory response, different changes to low density lipoprotein cholesterol (LDL-C) increases atherosclerotic events. Oxidation of small dense particles of LDL-C is more likely to occur to initiate atherogenesis (Ivanova et al., 2017).

LDL-C levels displayed significant elevation in mastitic cattle compared to healthy cattle. Higher levels of LDL-C can be attributed to hypertriglyceridemia which results in increased amounts of small, dense LDL particles and remnant lipoproteins due to accumulation of VLDL and disturbances in delipidation of VLDL and LDL (Packard et al., 2020). Elevated levels of LDL-C are also due to genetic defects that affect structure of apolipoprotein B of LDL and function of LDL receptors or polygenic disorders disturbing lipid metabolism (Freeman, 2010).

Dyslipidaemia is a significant risk factor for the development of CVDs, primarily characterised by high levels of low-density lipoprotein cholesterol (LDL-C) and decreased HDL-C concentrations (Poss et al., 2011).

VLDL-C contributes to the transportation of hepatic triacylglycerol to the adipose tissue (Satyanarayana and Chakrapani, 2006). VLDL-C remnants are significantly atherogenic due to their smaller size, high cholesterol concentration and proinflammatory properties because of their triglyceride concentration (Nordestgaard, 2016).

The VLDL-C levels in mastitis group showed significant elevation in current study. It may be due to excessive triglycerides accumulation in the mastitic cattle. Moreover, it can be

attributed to large amount of fatty acids in liver which results in higher TG levels and their secretion as VLDL cholesterol (Khovidhunkit et al., 2004).

CONCLUSION

These alterations in lipid profile possess serious threats in developing cardiovascular diseases in cattle suffering from mastitis, which results in sudden economic loss to the farmers. Hence, the results of this investigation can be helpful to minimize the economic risks associated with the mastitis infection. Moreover, it is mandatory to have regular lipid biomarkers analysis to minimize the cardiovascular risks in cattle infected with clinical mastitis.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

1. Abdel-Hamied E, Mahmoud MM (2020). Antioxidants profile, oxidative stress status, leukogram and selected biochemical indicators in dairy cows affected with mastitis. J. Anim. Health Prod. 8(4): 183-188.
2. Adesola, AE (2012). Antimicrobial resistance pattern of Streptococci and Staphylococci isolated from cases of bovine clinical mastitis in Nigeria. Nat. Sci. 10(11):96-101.
3. Adiels, M, S O Olofsson, M.-R Taskinen, and J Borén (2008). Overproduction of very low–density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. Arterioscler. Thromb. Vasc. Biol. 28(7):1225-1236.
4. Ali A, Mir BA, Bhat RR, Baba OK, Hussain SA, Rashid SM, Muzamil S, Ahmad SB, Mir MU (2017). Metabolic profiling of dairy cows affected with subclinical and clinical mastitis. J. Entomol. Zool. Stud. 5(6): 1026-8.

5. Ali KM, Wonnerth A, Huber K, Wojta J (2012). Cardiovascular disease risk reduction by raising HDL cholesterol–current therapies and future opportunities. Br. J. Pharmacol. 167(6): 1177-1194.
6. Arfuso F, Fazio F, Levanti M, Rizzo M, Di Pietro S, Giudice E, Piccione G (2016). Lipid and lipoprotein profile changes in dairy cows in response to late pregnancy and the early postpartum period. Arch. Anim. Breed. 59(4): 429-434.
7. Belay N, Mohammed N, Seyoum W (2022). Bovine mastitis: prevalence, risk factors, and bacterial pathogens isolated in lactating cows in Gamo zone, southern Ethiopia. Vet. Res. 9-19.
8. Borén J, Williams KJ (2016). The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. Curr. Opin. Lipidol. 27(5): 473-83.
9. Das D, Panda SK, Kundu AK, Jena B, Das BC, Sahu RK (2018). Haematological and metabolic profile test of mastitis affected bovines in Odisha. J. Entomol. Zool. Stud. 6(2): 3022-3024.
10. Esteve E, Ricart W, Fernández-Real JM (2005). Dyslipidemia and inflammation: an evolutionary conserved mechanism. Clin. Nutr. 24(1): 16-31.
11. Feingold KR, Grunfeld C (2010). The acute phase response inhibits reverse cholesterol transport 1. J. Lipid Res. 51(4): 682-684.
12. Fernández C, María del Val TL, Gómez-Coronado D, Lasunción MA (2004). Cholesterol is essential for mitosis progression and its deficiency induces polyploid cell formation. Exp. Cell Res. 300(1): 109-20.
13. Freeman MW (2010). Lipoprotein metabolism and the treatment of lipid disorders. Endocr. 788-807.
14. Friedewald WT, Levy RI, Fredrickson DS (1972). Estimation of the concentration of low-density

lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18(6):499-502.

15. Gross JJ, Schwarz FJ, Eder K, van Dorland HA, Bruckmaier RM (2013). Liver fat content and lipid metabolism in dairy cows during early lactation and during a mid-lactation feed restriction. J. Dairy Sci. 96(8): 5008-5017
16. Hameed S, Arshad M, Ashraf M, Avais M, Shahid MA (2012). Cross-sectional epidemiological studies on mastitis in cattle and buffaloes of Tehsil Burewala, Pakistan. J. anim. plant sci. 22(3): 371-376.
17. Hassing HC, Surendran RP, Mooij HL, Stroes ES, Nieuwdorp M, Dallinga-Thie GM (2012). Pathophysiology of hypertriglyceridemia. Biochim. Biophys. Acta - Mol. Cell. 1821(5): 826-832.
18. Hirayama S, Miida T (2012). Small dense LDL: an emerging risk factor for cardiovascular disease. Clin. Chim. Acta. 414: 215-224.
19. Ivanova EA, Myasoedova VA, Melnichenko AA, Grechko AV, Orekhov AN (2017). Small dense low-density lipoprotein as biomarker for atherosclerotic diseases. Oxid. Med. Cell. Longev. 2017.
20. Kalmath GP, Swamy MN, Yathiraj S (2013. Effect of summer stress and supplementation of vitamin E and selenium on serum lipid profile in Hallikar cattle. Int. J. Sci. Res. 4: 95-97.
21. Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C (2004). Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. J. Lipid Res. 45(7):1169-96.
22. Kovačić M, Samardžija M, Đuričić D, Vince S, Flegar-Meštrić Z, Perkov S, Gračner D, Turk R (2019). Paraoxonase-1 activity and lipid profile in dairy cows with subclinical and clinical mastitis. J. Appl. Anim. Res. 47(1):1-4.

23. Maxfield FR, Tabas I (2005). Role of cholesterol and lipid organization in disease. Nat. 438(7068): 612-21.
24. Nordestgaard BG (2016). Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease: new insights from epidemiology, genetics, and biology. Circ. Res. 118(4): 547-63.
25. Novac CȘ, Nadăș GC, Matei IA, Bouari CM, Kalmár Z, Crăciun S, Fiț NI, Dan SD, Andrei S (2022). Milk Pathogens in Correlation with Inflammatory, Oxidative and Nitrosative Stress Markers in Goat Subclinical Mastitis. Anim. 12(23): 3245.
26. Packard CJ, Boren J, Taskinen MR (2020). Causes and consequences of hypertriglyceridemia. Front. Endocrinol. 11: 252.
27. Pham CT (2006). Neutrophil serine proteases: specific regulators of inflammation. Nat. Rev. Immunol. 6(7): 541-50.
28. Poss J, Custodis F, Werner C, Weingartner O, Bohm M, Laufs U (2011). Cardiovascular disease and dyslipidemia: beyond LDL. Curr. Pharm. Des. 17(9): 861-70.
29. Qayyum A, Khan JA, Hussain R, Avais M, Ahmad N, Khan MS (2016). Investigation of milk and blood serum biochemical profile as an indicator of sub-clinical mastitis in Cholistani cattle. Pak. Vet. 36(3): 275-9.
30. Radostits OM, Gay C, Hinchcliff KW, Constable PD, editors (2006). Veterinary Medicine E-Book: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sci.
31. Ryman VE, Packiriswamy N, Sordillo LM (2015). Role of endothelial cells in bovine mammary gland health and disease. Anim. Health Res. Rev. 16(2): 135-49.
32. Sadashiv, SO, Kaliwal, BB (2014). Antibiotic resistance of Staphylococcus aureus and Coagulase-Negative Staphylococci (CNS) isolated from bovine mastitis in the region of north Karnataka, India.World J. Pharm. Res. 3: 571-586.

33. Sandesara PB, Virani SS, Fazio S, Shapiro MD (2019). The forgotten lipids: triglycerides, remnant cholesterol, and atherosclerotic cardiovascular disease risk. Endocr. Rev. 40(2): 537-557.
34. Sarvesha K, Satyanarayana ML, Narayanaswamy HD, Rao S, Yathiraj S, Isloor S, Mukartal SY, Singh SV, Anuradha ME (2017). Haemato-biochemical profile and milk leukocyte count in subclinical and clinical mastitis affected crossbred cattle. J. Exp. Biol. Agric. Sci. 5(1): 1-6.
35. Tabet F, Rye KA (2009). High-density lipoproteins, inflammation and oxidative stress. Clin. Sci. 116: 87-98.
36. Trinick TR, Duly EB (2005). Hyperlipidemia an Overview.
37. Turk R, Podpečan O, Mrkun J, Kosec M, Flegar-Meštrić Z, Perkov S, Starič J, Robić M, Belić M, Zrimšek P (2013). Lipid mobilisation and oxidative stress as metabolic adaptation processes in dairy heifers during transition period. Anim. Reprod. Sci. 141(3-4): 109-15.
38. Walker HK, Hall WD, Hurst JW (1990). Clinical methods: the history, physical, and laboratory examinations.

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Cervical Abnormalities are related to Infertility: A Review

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ABSTRACT: *Infertility is a disease and cervical abnormalities of the genital tract in females may cause this condition. To evaluate the cervical abnormalities related to infertility in females a systematic review was done. Related literature were collected via Google Scholar, NCBI, PubMed, and Medscape. For article searching following keywords were used: cervical abnormalities, infertility, uterine anomalies, and cervical factors. As the literature reviewed, more than 60 articles were studied, from which only 45 articles were added. The study suggested that many cervical abnormalities affect fertility including the poor interaction of mucus and sperm in the cervix, cervical cancer, dilatation and stenosis of the cervix, wall irregularities, diverticulum, masses, and metaplasia of the cervix. Some of the microorganisms also affect cervical functions causing infertility. It is concluded that cervical abnormalities can be related to infertility.*

Keyword: *Cervical abnormalities, infertility, uterine abnormalities, cervical factors*

INTRODUCTION

Infertility is described as a disease in which failure of getting pregnant is noted after unprotected regular intercourse for a couple of months (Shahzad et al., 2022). Normally, the term infertility is given to the couple for unsuccessful sex cycles after unprotected and regular intercourse (Wilcox et al., 2010). Some couples

take proper treatment to be pregnant, but they did not conceive, and some couples conceive naturally but late. There are two basic types of infertility including primary and secondary infertility. Infertility involved the genital organs of both males and females (John et al., 2022; Noor et al., 2021). Evaluation of infertility in females is necessary including uterotubal factor, ovulatory factor, and male factor (Carson et al., 2021).

Cervical Abnormalities

The cervix performs an intriguing gatekeeping function by first blocking infections from the vagina from ascending into the uterus and then by permitting sperm to ascend to the Fallopian tubes (Martyn et al., 2014). Additionally, it is essential for preserving the pregnancy in the uterus until labor starts. One of the important roles played by the female reproductive anatomy in fertilization includes the cervix because it involves the transportation of the sperms actively within the uterus (Elad et al., 2020). Any abnormality in the cervix can be related to infertility. In normal reproductive-age women, the mucus of the cervix is produced at the rate of 20 to 60mg/day and in the pre-ovulatory phase, this rate increases up to 700mg/day (Schmidt et al., 2013). This mucus of the cervix acts as a barrier for abnormal sperm. It contains secretions in small amounts of tubes, endometrium, and follicles. The unavailability of mucus can be the cause of infertility. More than a 200million sperms are deposited in the vaginal canal, of which only hundreds of sperms can reach the ovum (Mortimer et al., 2022; Swan et al., 2022). Little modification in the mucus can change the cervix structure which can lead to infertility and failure of natural pregnancy (Moghissi et al., 1972; Nakano et al., 2015). The diagnosis and treatment of cervical mucus abnormalities can lead to natural fertility. Different modalities are used to diagnose infertility including pelvic ultrasound, and MRI (Medicine et al., 2015). The test used for diagnosis involves an ultrasound

and postictal test (PCT), a valuable tool that can diagnose the cause of infertility in women. The treatment involves clomiphene therapy, which is the most common (Bloom et al., 2012; Boursicot et al., 2010; Check et al., 2021).

Microorganisms Causing Infertility

Some of the microorganisms including *Escherichia Coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* can affect cervical functions (Kaur et al., 1986). The early assessment of cervical or vaginal microflora can be useful in treating the cause of infertility and can enhance the chance of therapeutic protocols (Campisciano et al., 2017). The diagnosis can be achieved by laboratory tests including semen analysis, serum progesterone levels, and hormone levels in both genders (Makar et al., 2002). In infertility, the cervical factor counts for approximately 5% of all females (Assefa et al., 2019). The abnormalities of the cervix can be congenital and can be acquired in different females. Stenosis of the cervical canal involving internal os can lead to infertility (Zafarani et al., 2015). The stenosis can be due to polyp or endometriosis in the cervix. The early assessment of the stenosis can be useful for the therapeutic process of infertility (Moramazi et al., 2018).

Diagnosis of Cervical Abnormality

Another abnormality of the cervix that can affect fertility is the cervical collecting diverticulum, mostly it is congenital (Tanaka et al., 2020; Zafarani et al., 2015). The diagnosis can be via ultrasound, MRI, and hysterosalpingography (HSG) while its effective treatments include ovulation induction and ultrasound-guided intrauterine insemination (Carson et al., 2021; Sehring et al., 2021). HSG is an effective and less invasive tool to evaluate female reproductive organs. It is most widely used to rule out causes of infertility in females (Bajpai et al., 2014). It can easily detect cervical abnormalities including dilatation, stenosis, wall irregularities, diverticulum, and extra masses (Seoud

et al., 2002; Zafarani et al., 2015). One of the causes of cervical abnormalities leading to infertility is cancer of the cervix. In developing countries, it is very common to be affected by cervical cancer. Its etiology involves the Human papillomavirus (HPV) which can be spread sexually (Faridi et al., 2011; Jalil et al., 2020). The best method to diagnose this is screening. It can seriously affect fertility therefore there is a need for a cervical smear test for avoiding it actively (Nnadi et al., 2014). Trichomoniasis is another common cause of infertility. It is a sexually transmitted disease that occurs due to Trichomoniasis, a parasite (Menezes et al., 2016). The invasion of this parasite can lead to chromosomal modifications and causes cervical abnormalities. Females, positive with this parasite in the cervix remained infertile (Merdaw et al., 2018).

Epidemiology of Cervical Abnormalities

Worldwide, infertility may affect a couple of reproductive ages more commonly. Infertility affects 15% to 20% of American couples overall, with older couples experiencing higher rates. In Pakistan, 22% of women come to clinics due to the problem of infertility. The average age of most females is 29 years (Poon et al.,1985). Usually, at a yearly well-women screening, the female partner first seeks treatment for an infertility issue. This study will help a general practitioner to start the diagnostic examination and treat several infertility-related conditions.

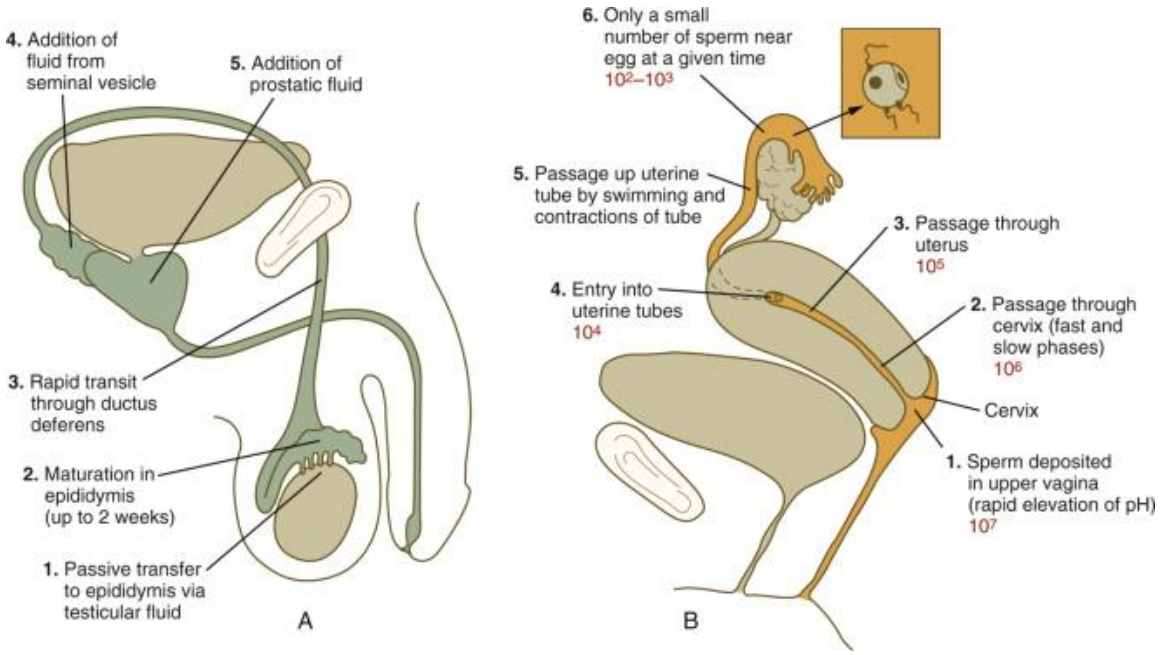

Fig. 1. Sperm transport in (a) the male and (b) the female reproductive tracts (Carlson, 2018)

METHODOLOGY

A literature review of 49 articles was performed with the use of search engines. The search engines that were taken for the study were Google scholar, NCBI, PubMed, and Medscape. The keywords that were used for searching were cervical abnormalities, infertility, uterine abnormalities, and cervical factors. Extraction of data is completely done from journal articles. As the literature reviewed, more than 60 articles were studied, and only 49 articles were added in this study that concluded cervical abnormalities are related to infertility.

Poor interaction of mucus and sperm affects Fertility

Fertility can be affected by the poor interaction of mucus and sperm in the cervix thus, the unavailability of cervical mucus can be the cause of infertility. One of the causes of cervical abnormalities leading to infertility is cancer of the cervix. The

dilatation and stenosis of the cervix, wall irregularities, diverticulum, and extra masses are the causes of cervical abnormality leading to infertility. Tosseous metaplasia of the cervix can lead to infertility in females.

The known cause of infertility can be the poor interaction of mucus and sperm. The increased rate of pregnancy in females is related to the mucus of the cervix. A low amount of cervical mucus indicates a low rate of sperm transportation and low ovum quality to fertilize (Fig. 1). Martyn et al. reviewed the cervix's function in fertility, published in 2014, and concluded with similar findings that fertility can be affected by the poor interaction of mucus and sperm in the cervix (Kyrgiou et al., 2014). Therefore, ovulation induction and intrauterine inseminations (IUI) both should be used to increase the rate of pregnancy in females having a low amount of cervical mucus (Farhi et al., 1995). This is an effective treatment and is very useful as stated by Soheila Akbar *et.al* study published in 2012. Couples who wanted to conceive should perform IUI with the husband's semen. It can also be performed in females with less infertility duration. This is a useful procedure in young females of an average age of fewer than 30 years (Zadehmodarres et al., 2009).

Certain microbes may have an impact on cervical function. Early detection of cervical microflora can help with therapy procedures and in treating the underlying cause of infertility. Laboratory testing can be used to make the diagnosis (Brouwer et al., 2010). Elevated cervical levels of IL-1 and IL-8 can cause bacterial vaginosis (BV) that can affect fertility in females as stated by P Mastromarino et.al study's published in 2014 (Spandorfer et al., 2001). A prospective study was conducted in a University hospital to rule out the effect of preoperative and postoperative effects of the septate uterus, and the duplicate uterus on infertility (Valle et al., 2013). It was concluded that the spare of the duplicate cervix and hysteroscopic

septum resection (HSR) can lead to the increased thickness of the internal OS of the cervix causing cervical stenosis and further leading to infertility (Wang et al., 2009). Cervical collecting diverticulums are another malformation of the cervix that may have an impact on fertility. Hysterosalpingography, MRI, and ultrasound can all be used to make the diagnosis (HSG) as stated by NJ Khati et al in their study published in 2012. Osseous metaplasia of the cervix can lead to infertility in females. Deep osseous metaplasia in the cervix can cause uterine perforations to become a major cause of infertility. It can be diagnosed by using TVS (transvaginal ultrasonography). Therapeutic processes may include laparotomy and hysterectomy (Polat et al., 2011).

Cervical Cell Lesions and Infertility

The causes that are seen in females are mostly cervical cell lesions that are causing infertility. The PAP smear is performed that is more sensitive for this purpose. Precancerous lesions can also cause infertility (Sachan et al., 2018). According to the National Cancer Registry (NCR) of Norway, a study concluded that precancerous lesions in the fallopian tubes, uterus, and cervix can cause infertility (Holst et al., 1983). Sexually transmitted diseases including cervical cancer are more common to cause infertility in females. A cross-sectional study was performed in Nigeria concluding that epithelial cell abnormalities in the cervix lead to infertility but infertility itself did not increase the rate of cervical changes in the females (Mbazor et al., 2011). Infertility can be of unknown cause which is called unexplained infertility. In this type, the cause of infertility cannot be detected in both males and females. But searches are being performed to know the relation between cervical abnormalities with unexplained infertility (Prabha et al., 2011).

CONCLUSION

It was concluded from the review study that different cervical abnormalities are related to infertility in females.

CONFLICT OF INTEREST

Authors declared there is no conflict of interest.

REFERENCES

1. Assefa AA, Astawesegn FH, Eshetu B (2019). Cervical cancer screening service utilization and associated factors among HIV positive women attending adult ART clinic in public health facilities, Hawassa town, Ethiopia: a cross-sectional study. BioMed Cent. 19 (1): 1-11.
2. Bajpai T, Bhatambare G, Shrivastava G (2014). Simple non-invasive and cost-effective method for detection of Chlamydia trachomatis infection (a silent, sexually transmitted pathogen that can cause infertility). Int. J. Health Allied Sci. 3 (1): 66.
3. Bloom S, Webster G, Marks D (2012). Oxford handbook of gastroenterology and hepatology: Oxford university press.
4. Boursicot K, Sales D (2010). Clinical Specialties: Oxford University Press.
5. Brouwer MC, Tunkel AR, Van de Beek D (2010). Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. Clin. Microbiol. Rev. 23 (3): 467-492.
6. Campisciano G, Florian F, D'Eustacchio A (2017). Subclinical alteration of the cervical–vaginal microbiome in women with idiopathic infertility. J. Cell. Physiol., 232 (7): 1681-1688.
7. Carlson BM (2018). Human embryology and developmental biology. Elsev. Health Sci.
8. Carson SA, Kallen AN (2021). Diagnosis and management of infertility: a review. Jama. 326 (1): 65-76.
9. Check J (2021). Diagnosis and treatment of cervical mucus abnormalities. CEOG. 33 (3): 140-142.

10. Elad D, Jaffa, AJ, Grisaru D (2020). Biomechanics of early life in the female reproductive tract. Physiol. 35 (2): 134-143.
11. Farhi J, Valentine A, Bahadur G (1995). In-vitro cervical mucus—sperm penetration tests and outcome of infertility treatments in couples with repeatedly negative post-coital tests. Hum. Reprod. 10 (1): 85-90.
12. Faridi R, Zahra A, Khan K (2011). Oncogenic potential of Human Papillomavirus (HPV) and its relation with cervical cancer. Virol. J. 8 (1): 1-8.
13. Holst, N, & Abyholm T (1983). Precancerous lesions of the cervix uteri in infertile women. Br Med J (Clin Res Ed), 287 (6398), 1019-1020.
14. Jalil AT, & Karevskiy A (2020). The cervical cancer (CC) epidemiology and human papillomavirus (HPV) in the middle east. Int. J. Environ. Eng. 2 (2): 7-12.
15. John A, Faridi TA (2022). Awareness and Knowledge of Human Immunodeficiency Virus Transmission and Prevention from Mother to Child: A Cross-Sectional Study among Female Sex Workers: HIV Transmission and Prevention from Mother to Child. PBMJ: 54-58.
16. KAUR M, Tripathi K, Bansal M (1986). Bacteriology of cervix in cases of infertility: effect on human sperm. Am. J. Reprod. 12(1): 21-24.
17. Kyrgiou M, Mitra A, Arbyn M, (2014). Fertility and early pregnancy outcomes after treatment for cervical intraepithelial neoplasia: systematic review and meta-analysis. Bmj. 349.
18. Makar RS, Toth TL (2002). The evaluation of infertility. Pathol. Patterns Rev. 117(1): S95-S103.
19. Martyn F, mcauliffe F, & Wingfield M (2014). The role of the cervix in fertility: is it time

for a reappraisal? Hum. Reprod. 29 (10): 2092-2098.

20. Mbazor J, Umeora O, Egwuatu V.(2011). Cervical cytology profile of infertility patients in Abakaliki, South-eastern Nigeria. JBJOG. 31 (2): 173-177.
21. Medicine PCOTASFR (2015). Diagnostic evaluation of the infertile female: a committee opinion. Fertil. Steril. 103(6): e44-e50.
22. Menezes CB, Frasson AP, & Tasca T (2016). Trichomoniasis-are we giving the deserved attention to the most common non-viral sexually transmitted disease worldwide? Cell. Microbiol. 3 (9): 404.
23. Merdaw M, Kadhim HS, Abd alsattar Alriyahee F (2018). Genetic variation of Trichomonas vaginalis isolates from Iraqi Women: Association with fertility and cervical abnormalities. JUBPAS. 26 (7): 321-338.
24. Moghissi KS (1972) The function of the cervix in fertility. Fertil. Steril. 23(4): 295-306.
25. Moghissi KS (1987). Cervical and uterine factors in infertility. Obstet. Gynecol. Clin. North Am. 14(4): 887-904.
26. Moramazi F, Roohipoor M & Najafian M (2018). Association between internal cervical os stenosis and other female infertility risk factors. Middle East Fertil. Soc. J. 23(4): 297-299.
27. Mortimer D, Björndahl L, Barratt CL (2022). A practical guide to basic laboratory andrology: Cambridge University Press.
28. Nakano FY, Leão, RD (2015). Insights into the role of cervical mucus and vaginal ph in unexplained infertility. Medicalexpress, 2.

29. Nnadi D, Nwobodo E, Ekele B (2014). Screening for cervical cancer: A review of outcome among infertile women in a tertiary hospital in North-West Nigeria. Ann. Med. Health Sci. Res. 4 (3): 383-387.
30. Noor S, Rana MS, Hanif A (2021). Determinants of Lack of Family Planning in Grand Multiparous Women: Lack of Family Planning in Grand Multiparous Women. PBMJ, 4 (1).
31. Polat I, Sahin O, Yildirim G (2011). Osseous metaplasia of the cervix and endometrium: a case of secondary infertility. Fertil. Steril. 95 (7): 2434. E2431-2434. E2434.
32. Poon WW, Mccoshen JA (1985). Variances in mucus architecture as a cause of cervical factor infertility. Fertil. Steril. 44(3): 361-365.
33. Prabha V, Aanam TD, Kaur S (2011). Bacteriological study of the cervix of females suffering from unexplained infertility. Am. J. Biomed. Sci., 3(2).
34. Sachan PL, Singh MPatel, ML (2018). A study on cervical cancer screening using pap smear test and clinical correlation. Asia-Pac. J. Oncol. Nurs. 5 (3): 337-341.
35. Schmidt CL (2013). In vitro fertilization. Human in Vitro Fertilization and Embryo Transfer. 59.
36. Sehring J, Hussain A, Beltsos, A, (2021). Role of Imaging in the Management of Female Infertility Breast Gynecological Diseases (pp. 441-463): Springer.
37. Seoud M, Awwad J, Adra A, (2002). Primary infertility associated with isolated cervical collecting diverticulum. Fertil. Steril. 77(1): 179-182.
38. Shahzad H, John A, Ali A (2022). Incidence of infertility in females and Evaluation of its Causes Using Ultrasonography: Incidence and Causes of

Infertility in Females. PBMJ, 55-58.

39. Spandorfer SD, Neuer A, Giraldo PC (2001). Relationship of abnormal vaginal flora, proinflammatory cytokines and idiopathic infertility in women undergoing IVF. J. Reprod. Med. 46 (9): 806-810.
40. Swan SH, Colino S (2022). Count down: How our modern world is threatening sperm counts, altering male and female reproductive development, and imperiling the future of the human race: Simon and Schuster.
41. Tanaka K, Shiga N, Kuno T, (2020). Successful pregnancy and vaginal delivery after laparoscopic excision of a congenital uterine cervical diverticulum: A case report. J. Obstet. Gynaecol. 46(8): 1460-1464.
42. Valle RF, Ekpo GE (2013). Hysteroscopic metroplasty for the septate uterus: review and meta-analysis. J. Minim. Invasive Gynecol. 20 (1): 22-42.
43. Wang JH, Kai-Hong, X Lin J (2009). Hysteroscopic septum resection of complete septate uterus with cervical duplication, sparing the double cervix in patients with recurrent spontaneous abortions or infertility. Fertil. Steril. 91 (6): 2643-2649.
44. Wilcox AJ (2010). Fertility and pregnancy: an epidemiologic perspective: Oxford University Press.
45. Zadehmodarres S, Oladi B, Saeedi S (2009). Intrauterine insemination with husband semen: an evaluation of pregnancy rate and factors affecting outcome. J. Assist. Reprod. Genet. 26(1): 7-11.
46. Zafarani F, Ahmadi F, Shahrzad G (2015). Hysterosalpingographic features of cervical abnormalities: acquired structural anomalies. Brit. J. Radiol. 88(1052): 20150045.
47. Bruce M.Carlson MD, PhD, in The Human Body. 2019
48. Krysiewicz S (1992). An overview. AJR 50(5): 314-319.

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Evaluation of Zoological Gardens in Punjab in Public Education and Captive Wildlife Conservation

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ABSTRACT: *The evolution of methods for keeping wild animals in captivity can be traced back many centuries. The desire to keep animals in captivity for recreational purposes led to the development of several zoological parks after man adopted a more settled way of life. These zoos, aquariums, and other animal sanctuaries must now serve a more serious purpose, one that goes beyond entertainment. Around 700 million people visit zoos and aquariums each year, as reported by WAZA (World Association of Zoos and Aquariums). True zoos and aquariums provide special opportunities for local communities to get involved in protecting wildlife. In addition, zoological parks are widely acknowledged for the invaluable contributions they make to conservation and scientific study by means of the animals and plants in their living collections. This research was devised to evaluate the conservation and educational impact of the Lahore Zoological Gardens, the Bahawalpur Zoological Gardens, and the Marghazar Zoological Gardens. In general, the results showed that the Lahore Zoo was the best of the selected zoos. However, for a variety of reasons (including a lack of a zoo animal keeper training programme, poor record keeping, inbreeding, inadequate housing, inadequate veterinary care, an inadequate animal collection plan, the absence of an on-site animal nutritionist, and a lack of or improper public education and awareness programming), the Lahore Zoo did not meet WAZA standards so far.*

Keyword: *Captive wildlife , Zoological Gardens, Education, Conservation, WAZA*

INTRODUCTION

Zoos and aquariums have evolved in recent times so that they can better serve as locations for wildlife control (Carr and Cohen, 2011). Today's zoos and aquariums serve a far broader purpose of educating the public and ensuring the well-being of exotic species. Although zoos had humble beginnings as animal exhibition halls and tourist attractions, they made great strides in the 1800s. Existing legislation protects wildlife and prioritises their well-being (Whitworth, 2012). Today's zoos and aquariums show the public a world that has been intentionally preserved for their safety. Zoos should display what gardens, parks, and cities could look like if steps were done to improve water quality by laying a green foundation, implementing good biological systems without disturbing species, and providing habitat for local, diverse flora.

The role of zoos in animal research and biodiversity conservation has grown in importance over the years, with many zoos now advocating for comprehensive strategies to protect endangered species, such as the One Plan Approach (Minteer et al., 2013). By bringing together different types of conservationists (like field researchers, zookeepers, wildlife managers, and other veterinary specialists) This conservation technique in which zoos play a key role helps to bridge the gap between wild and captive population management to produce a unified planning tool for the preservation of species. Everyone who visits a zoo should understand that doing so helps save animals in their natural habitats. Local, provincial, and state governments are the primary actors in this context. The media and environmental organisations are also crucial players (Wagner et al., 2009).

The public's opinion of a zoo can be gauged by looking at how well it is known and respected, all of which are aspects of the institution's reputation. Reputation is a multifaceted concept, but by illuminating the most important variables that can have a negative impact on zoos' reputations, we can begin to discover strategies to improve

it. In order to solve these pressing problems, zoos must advocate for more humane practices in animal care and management and work to increase the awareness of the significance of their mission among key constituents. The public's willingness to financially support zoos in the future as centers for biodiversity conservation and educational and entertaining venues for the environmental community will gain from this, as will the individual zoos themselves (Ajayi and Tichaawa, 2021).

Many kinds of people from all over the world flock to visit zoos. As a popular recreational destination, zoos play an important role in the lives of Pakistanis. Punjab Wildlife & Parks Department manages two of the province's three zoos—specifically, the Lahore Zoological Gardens and the Bahawalpur Zoological Gardens. Marghzar Zoological Gardens in Islamabad, which is managed by the Capital Development Authority (Capital Development Authority). Research is currently being conducted at these zoos. The Lahore Zoo, which opened in 1872, is the world's first. (web.1). Located on a 25-acre plot of land, the Bahawalpur Zoological Gardens first opened to the public in 1942. It is well-known for its efforts to preserve and breed lions (web.2). Marghazar Zoo was established in 1978 and spans an area of 82 acres. It was originally created to provide protection and food for local spotted deer, leopards, and Indian gazelles. The World Association of Zoos and Aquariums (WAZA) has joined this international organisation (WAZA, 2005; 2022). Its membership includes more than 300 institutions from throughout the world, including aquariums, zoos, cooperation partners, and related organisations. Its primary goal is to facilitate coordination between zoos and aquariums around the globe in order to better protect, manage, and breed animals in captivity. In 2007 (Raja, CZA). Unfortunately, no zoos in Pakistan are part of WAZA, and the only Asian members are Dusit Zoo in Bangkok and Central Zoo Authority. This research was designed to assess the

contribution of zoos in Punjab, Pakistan, to conservation and education in line with initiatives promoted by WAZA. As mentioned earlier WAZA promotes the highest standards of care for animals in human care, including the management of endangered species and proper husbandry. In the case of animal conservation and protection, Zoos in Pakistan should improve their animal care strategies and try to be at par with WAZA guidelines. This study would be helpful to highlight the shortcomings for a better future of Zoo exhibits. Important for future national and international cooperation, the results will be improved interlinking and the exchange of knowledge and skills among the selected zoos.

MATERIALS AND METHOD

This project's study approach, based on a synthesis of literature, observations, precedent studies, and site analysis, aims to assess the contributions of various zoos to education and conservation in Punjab, Pakistan. As all of these techniques are tailored to the requirements of the researcher, animals, and visitors, they are all good choices.

Different questionnaires (Spiriti etal.,2022). were created to evaluate how people felt about the zoo, the management, the veterinarian care, and other animal welfare/conservation/educational services.

i. Precedent Study Methods

Educational programmes and design factors for animals, staff, and visitors in existing zoos were identified through a series of precedent studies. Analyzing relevant examples can shed light on problems, reveal avenues for improvement, and reveal hidden possibilities. It was attempted to use the WAZA's accreditation as a benchmark for researching antecedents for overall zoos and their educational programmes. Specifically, four examples were chosen that addressed the zoo's teaching programme, animal care, conservation efforts, and the overall experience of the zoo's visitors.

ii. Observation Methods

To gauge how many people visited, how long they stayed, whether or not they read the exhibit's signage, and whether or not the animals in the exhibit were active, it was essential to conduct observations. The Lahore Zoological Gardens, the Bhawalpur Zoological Gardens, and the Marghazar Zoo also participated in preliminary observational research. It was vital to check out the path leading up to the viewing area, the enclosure, and how close guests may go to the animals before entering the viewing area. Visitor reactions to educational programmes and conservation of wildlife at the zoos were also documented.

iii. Interview Method

Interviews with the directors, veterinarians, education officers and keepers of selected Zoos were conducted. The Table-1 details the results of the comprehensive education and conservation monitoring that followed the guidelines established by WAZA in 2005.

iv. Schedule of Interview with Visitors

Between the months of June 2020 and August 2020, researchers at Lahore Zoo and those at Marghazar Zoo gathered data. In the same year, during September and October, the Bahawalpur Zoo was also visited for this reason. Every day, on the weekends, and especially on public holidays, visitors were handed a questionnaire to fill out. The primary objective was to gather the opinions of a diverse cross-section of the people. The opinions of several uneducated tourists were sought out through interviews.

v. Diet Plan Analysis

Information on the animal food was gathered from zoo administration and veterinarians. Individuals at all three study locations provided their own feedback on the dietary regimens they were given. To ensure consistency in animal nutrition, the Lahore Zoo's feeding plan was used by all captive facilities. Many aspects of feeding schedules, diets, methods of food presentation and distribution,

individual animal preferences, the introduction of novel objects, the cleanliness of feeding areas, and the design of enrichment plans and enclosures were all recorded. Food amount and quality were also evaluated.

RESULTS AND DISCUSSION

One hundred surveys and interviews were conducted at each of the study sites. Figures 1a–1d provide a graphical representation of the feedback we received from our site users.

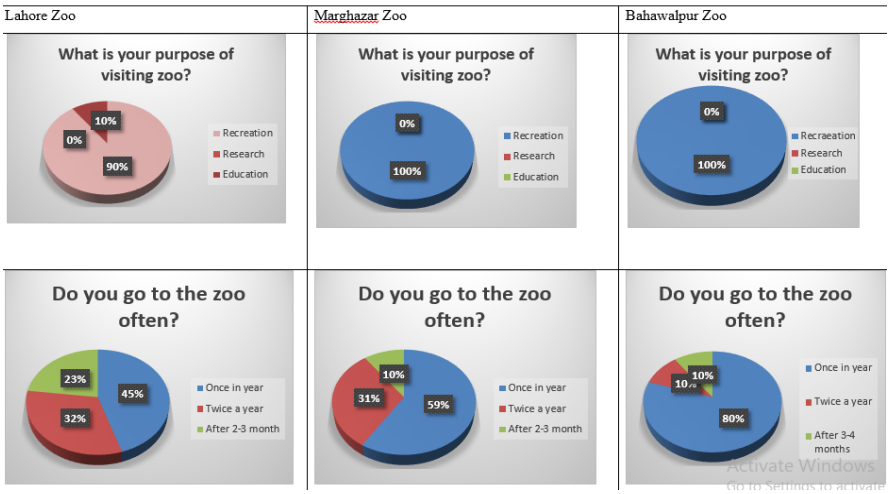

Fig. 1a. Results of survey conducted in different Zoos

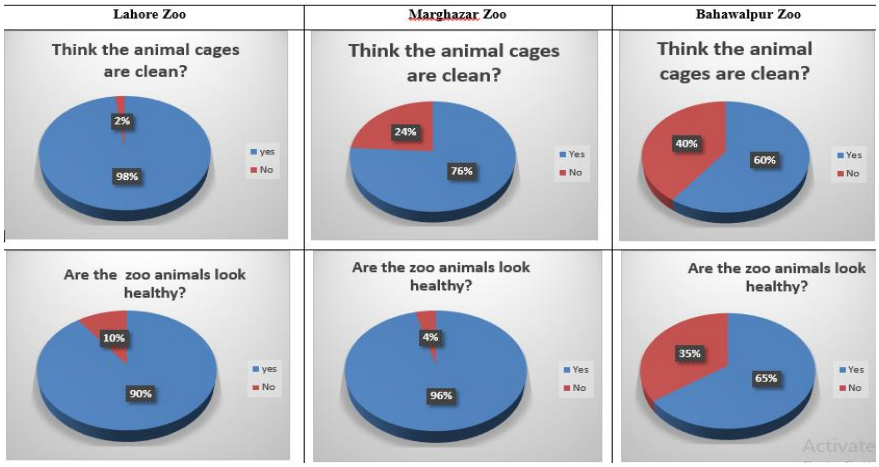

Fig. 1b. Results of survey conducted in different Zoos

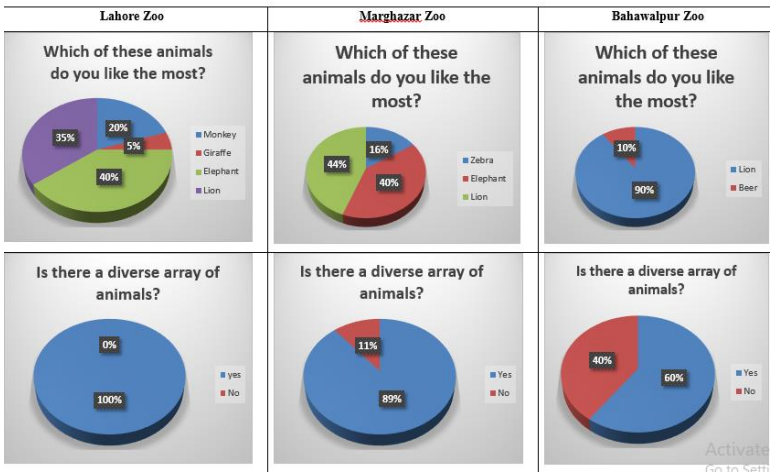

Fig. 1c. Results of survey conducted in different Zoos

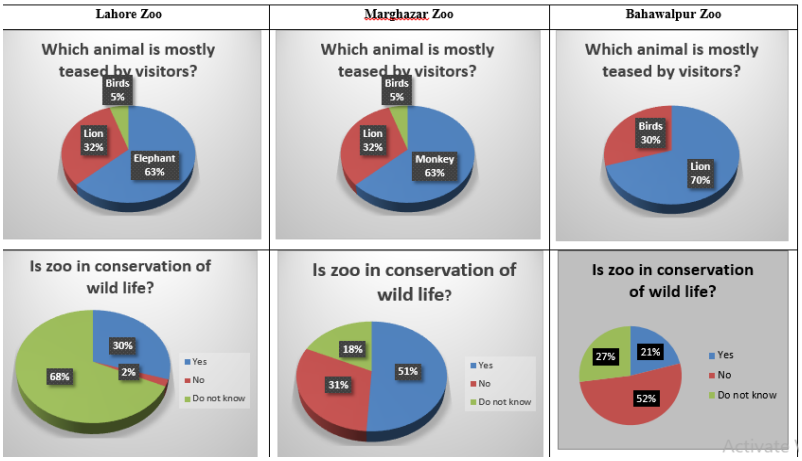

Fig. 1d. Results of survey conducted in different Zoos

Visitors' reactions were consistent with those reported by Hassan (2015). After comparing Lahore Zoo to other captive facilities, it was determined that it performed best in terms of conservation and education. The Lahore Zoo had superior accommodations for the animals. Two other zoos were directly emulating its enclosure style and construction materials. When compared to other captive settings, Lahore Zoo performed exceptionally well in every category, including animal food, habitat design, and medical treatment. Also, Lahore zoo had superior

veterinary facilities, and veterinarians are a crucial cog in the animal welfare wheel (Deem, 2007). Marghzar Zoo, however, provides a superior food for its animal residents. Bahwalpur Zoo's housing conditions were not unlike those of antiquated menageries, and as a result, animal health was severely impaired. Current research results also show that visitors prefer to contact with animals or like the animal facilities where they get chance intract with animal and note their behaviours this study is similar to findings of Ajayi & Tichaawa,2021 who analysed the connections between zoo visitors' levels of contentment, their sense of attachment to the zoo, and their dedication to the institution. There were six major takeaways presented. To begin, only place social bonding (and not any of the other four sub-constructs of place attachment) was found to be significantly influenced by visitors' satisfaction. As gathering places (Smith et al., 2012; Ajayi, 2019), zoos provide opportunities for people to learn about and appreciate nature together. Groups of tourists were seen learning, touring, and enjoying themselves while visiting the zoo, whereas solitary visitors actively sought out company.

As shown in figure 1-12 the 90% to 100% objective of zoo visit was recreational at all survey sites. The result about the question " which is your favourite animal" 90% and 44% people loved lions in Bahawalpur Zoo and Marghazar Zoo respectively while in Lahore Zoo 40% people still liked elephant and missing SUZI (Lahore Zoo old Female Elephant) these findings are in agreement with Carr, (2016).

Public survey results in the present study corroborate the previous conclusion that Lahore Zoo is more popular among tourists for recreational purposes than any of the other two zoos. People on their day off came here to have some fun. Simply put, a well-thought-out strategy for animal collection (Andrew and Maggie, 2010). The public has actually called for more animals, showing complete disregard for their well-being. Most people visiting the zoo had no idea of

endangered animals, conservation, or the difference between in-situ and out-of-situ conservation. Nothing about them promotes conservation education or public interest in zoo displays (Carr and Cohen, 2011). The Lahore Zoo has a well-established education programme but the result reveals that the response of people about the question “Does the zoo provide visitors with information on how to save endangered species?. Many visitors intensely disagreed with the statement that zoos teach tourists about wildlife conservation. again the findings are in agreement with Ajayi and Tichaawa, (2021). In this case it recommended that the awareness activities of zoo should be promoted on social media for general public information.

As far as the question “ how often you visit the zoo is concerned 80% of respondents at Bahawalpur Zoo said once in the year, more the half of visitors 59% at Marghazar Zoo also visited once in the year whereas, 45% visitors of Bhawalpur zoo visited once in the year as shown in the figure.

The Lahore zoo's administration also funds internships and research programmes in unique ways compared to other zoos. The finding of surveys suggested that people are unaware of the fact and it is not understandable this question that “Do zoos conduct research?” they did not show any definite view point. Interestingly, more than half of visitors(51%) at Marghazar Zoo said yes on question " Do you know that zoo involve in wildlife conservation projects?" whereas the only (30%) of Lahore Zoo-goers were agreed to it as shown in below Figures (1a-4d).

Only trustworthy zoos will have the visitors, community, and social

structures necessary to protect and educate about biodiversity. Because of this, it is crucial for zoo organisations and individual zoos to understand which aspects of their operations have the greatest potential to shape their reputations (Paxton et al., 2020). Indeed, such endeavours need a well-thought-out strategy for marketing and suitable commercialization. That, as noted by Ahmad et al., 2015, as well as

others, would pique the public's attention, which would lead to the creation of finances to ensure the future of such activities. As compared to the other zoos considered, the results showed that Lahore's was the best overall (Table 1), although it still fell short of WAZA's 2005 and 2022 requirements for a number of reasons.

Table 1: Captive Animal Conservation

Questions	**Lahore Zoo**		**Marghazar Zoo**		**Bahawalpur Zoo**	
	Yes	**No**	**Yes**	**No**	**Yes**	**No**
Have you, as a zoo administration, made any efforts to educate guests about animal welfare?	✓		✓		✓	
Is the zoo making any effort to ensure the animals have suitable living conditions?	✓		✓			✓
Is there any way to lessen the strain that tourists have on animals?	✓		✓			✓
Are the fences adequate for protecting the animals and the people who visit?	√			✓	✓	
Is there a plan in place for a crisis?	✓		✓		✓	
Is the zoo letting visitors know which animals are ill or hurt?		✓		✓		✓
Does the zoo have a formal, approved master plan?	✓			✓		✓
Is the exhibit's container built to meet its biological needs?	✓		✓		✓	
Is there a facility where endangered animals are housed that was built without official permission?		✓		✓		✓

How well the animals here are housed in accordance with their natural needs.		√		√	✓	
Is there a system in place to ensure that cooking equipment is regularly cleaned?	✓		✓		✓	
Is there a system in place to ensure that cooking equipment is regularly cleaned?	✓		✓		✓	
Feed offered to animals are according to nutritional requirement of animals?	✓		✓		✓	
The feed given to animals meets their nutritional needs?	✓		✓		✓	
Where can they go to get away, relax, and have babies?	✓		✓		✓	
Is the substrate and plant life in the enclosure similar to what you would find in a more natural setting?	✓		✓		✓	
Do you supply seasonal vitamin and mineral supplements to the animals?	✓		✓		✓	

Lack of budget for education and awareness.

- Lack of trained human resources
- Lack of zoo animal keeper training programme
- Lack of record keeping
- Inbreeding problems
- Improper housing facilities
- Lack of veterinary care facilities
- Lack of animal enrichment plan
- Improper animal collection plan
- Lack of animal nutritionist at zoo
- Same animal ration scale for every zoo
- Lack of modern visitor facilities
- Lack or improper public education and awareness programme
- Zoos are meant for recreation and money generation rather than conservation and education.

CONCLUSION

It was concluded on the basis of the current study that the major purpose of people visiting the zoo is entertainment but they also love to be part of animal base activities. Zoo is a family place and kids are the future ambassadors of wildlife and nature conservation among all selected zoos Lahore Zoo was much liked and in line with the aims and objectives of modern zoos. The Lahore Zoo can make its message more impactful if they share its zoo activities on social media to inform the public. Other zoos like Marghazar Zoo and Bahawalpur Zoo should improve their existing awareness and animal care programme for the betterment of captive wildlife.

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ETHICAL APPROVAL

The study was approved by the intuitional ethical review committee.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

1. Ahmad S, Ali Z (2015). The study of public perception for captive animals at Lahore Zoo Pakistan. J. Anim. Plant Sci. 25(3): 20
2. Ajayi O, Tichaawa T (2021). Exploring the relationships between satisfaction, place attachment and loyalty in Nigerian Zoos 37(1): 861–872.
3. Ajayi O (2019). Environmental attitudes, motivation and place attachment of visitors to federal institutionalized zoological gardens in the South-west, Nigeria. PhD thesis, University of Ibadan , Nigeria. pp 385
4. Andrew M (2010). Visitor interest in zoo animals and the implications for collection planning and zoo education programmes. Zoo Biol. 29(6): 715-731.

5. Carr N, Cohen S (2011). The public face of zoos: Images of entertainment, education, and conservation. Anthrozoos. 24(2): 175-189.
6. Carr N (2016). An analysis of zoo visitors' favourite and least favourite animals Tourism ManagementPerspectives. 70-76.
7. Deem S (2007). Role of the zoo veterinarian in the conservation of captive and free-ranging wildlife. Int. Zoo Yb. 41: 3–11.
8. Hassan K (2015). Measuring Visitors' Observation and Perception on Animal Welfare in National Zoo. Mediterranean Journal of Social Science. MCSER Publishing, Rome-Italy . Vol: 6 S2
9. IUCN (2015) The IUCN Red List of Threatened Species. Gland and Cambridge: IUCN. http://www.iucnredlist.org
10. Mooney A (2020). A system wide approach to managing zoo collections for visitor attendance and in situ conservation. Nat Commun 11: 584.
11. Minteer BA (2013). Ecological ethics in captivity: Balancing values and responsibilities in zoo and aquarium research under rapid global change. ILAR J. 54: 41–51.
12. Paxton P, Velasco K, Ressler RW (2020) . Does Use of Emotion Increase Donations and Volunteers for Nonprofits? Am. Sociol. Rev. 2020, 85: 1051–1083.
13. Raja A (2007). Zoo in India, legislation, Policy, Guidelines and strategy Central Zoo Authority.
14. Smith L, Weiler B (2012). Applying visitor preference criteria to choose pro-wildlife behaviors to ask of zoo visitors. Curator. 55: 453-466.
15. Spiriti MM (2022). Development of A Tool for Assessing the Reputation of Zoos: The Zoo Ethical Reputation Survey (ZERS). Anim. 12: 2802.
16. online: https://www.waza.org/ (accessed on 14 September 2022).
17. Wagner K, Chessler M (2009). Development and implementation of an evaluation strategy for measuring conservation outcomes. Zoo Biol. 28(5): 473-487.
18. WAZA (2005).Building a Future for Wildlife – The World Zoo and Aquarium Conservation Strategy, WAZA, Berne. ISBN 3-033-00427-X
19. Web.1:http://www.lahorezoo.com.pk/
20. Web.2http://www.mybahawalpur.com/bwp/zoo.html.

21. Whitworth, A.W. (2012) An Investigation into the Determining Factors of Zoo Visitor Attendances in UK Zoos. PLoS ONE 7(1): e2983.