CROSS ANTIGENICITY OF *SALMONELLA TYPHI* AND NOVEL CORONAVIRUS ANTIBODY IN SECOND WAVE OF COVID-19 PANDEMIC

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ABSTRACT

During second wave of COVID-19 pandemic, increase in incidence of typhoid was observed in different cities of Pakistan. Rapid diagnostic tests for COVID-19 and typhoid are less sensitive and confirmatory tests are required to diagnose the infection. Moreover, COVID-19 IgM mimic *Salmonella typhi* IgM and have same clinical presentations as typhoid. Muzaffargarh is a district of province Punjab. Being a hotspot for COVID-19, it also has high prevalence of Typhoid. Therefore, in this study we aim to evaluate the cross antigenicity of COVID-19 IgM with *Salmonella typhi* IgM. 593 patients were enrolled in study with informed consent. Blood samples were collected from patients and laboratory biomarkers were analyzed. Data was recorded and statistical analysis was done. Among study participants, 64% were males while 36% were females. All the laboratory biomarkers were elevated in all the patients. Different age groups didn't exhibit difference in all laboratory biomarkers except ferritin. Significant difference was observed in creatinine, LDH and ferritin levels in male and female patients. It can be concluded that all age groups are under same risk. However, disease severity is higher in male population.

Key words: COVID-19, typhoid, cross antigenicity, IgM, Muzaffargarh

INTRODUCTION

COVID-19 being a global pandemic had been affecting people worldwide since 2019. According to WHO, about 1,535,144 cases were reported from January 2020 to June 2022 in Pakistan. In middle- and low-income countries including Pakistan, healthcare system is severely burdened both in case of diagnosis and treatment.Common clinical symptoms of COVID-19 include fever, cough, diarrhea, dyspnea, rhinorrhea, headache and fatigue(Pascarella et al., 2020).Clinical representation of COVID-19 coincides with other diseases which make diagnosis biased and difficult. Moreover, most rapid tests used for COVID-19 diagnosis are less sensitive.

During second wave of COVID-19 pandemic (starting from 28 October, 2020)(Ali, 2020), increase in incidence of typhoid indicated the alarming situation in Pakistan when healthcare system was already suffering from COVID-19 cases. Typhoid is a bacterial infection caused by *Salmonella Typhi*. Common clinical symptoms of typhoid include fever, headache, loss of appetite, constipation, nausea or diarrhea. These symptoms are less specific and non-distinguishable in case of other infections(Ahmad et al., 2021). As typhoid fever has some common symptoms as indicated in case of COVID-19, suspicion of typhoid fever resulted into recommendation of typhi-dot or Widal test. These diagnostic tests are less sensitive and less specific. Moreover, COVID-19 IgM is also less specific and give positive result when tested for typhi-dot or Widal test. These conditions make diagnosis unclear and biased. So, recommended confirmatory tests are required for proper diagnosis.

Muzaffargarh is a district located in Punjab, comprising of five tehsils. During second wave of COVID-19 pandemic, Muzaffargarh was also indicated as COVID-19 hotspot as represented in Fig.1. According to National Institute of Health (NIH), there is only one laboratory having testing facility for COVID diagnosis. So, in this study we aim to evaluate the cross antigenicity of *Salmonella Typhi* IgM and novel coronavirus IgM in 2nd wave of COVID-19 pandemic in district Muzaffargarh.

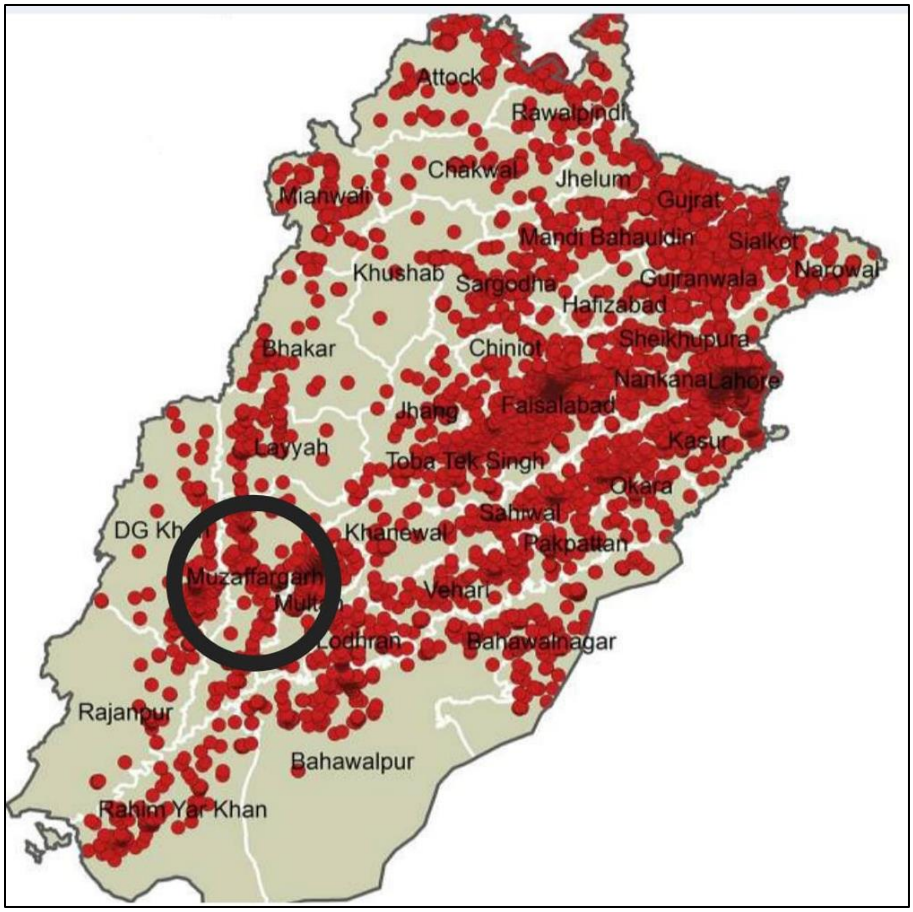

Fig. 1. Most affected area in Punjab reported with COVID-19 cases on 20 October, 2020 (Saeed *et al.*, 2021)

METHODOLOGY

This cross-sectional study was conducted in district Muzaffargarh. Data from 593 patients that were positive for typhoid IgM and COVID-19was obtained after informed consent. Patients suffering from other diseases were excluded from the study.

Sample collection and testing

Blood samples were collected from the patients that were positive for typhidot and COVID-19. Samples were processed for further testing. Total leucocyte count (TLC) was done using hematology analyzer. Creatinine and ALT were calculated using Biolabo kits 80107 and Spectrum 292002 kit respectively. C-reactive protein (CRP) value was obtained by using Rapid kit. While Ferritin levels were calculated by using Abbot ARCHITECT i1000SR immunoassay analyzer.

Statistical Analysis

Data was entered in Excel file and analyzed by using QuickCalcs of GraphPad software 8.0.1. And represented in percentages or mean with standard deviation. One way ANOVA was applied to check the difference in mean among different groups. Un-paired t-test was applied to check the difference between two independent groups. P value < 0.05 was considered significant.

RESULTS

Demographic details of the participants

Five hundred and ninety-three (593) patients were included in the present study. Among 593 participants, 381 (64%) were male while 212 (36%) were female. Age of study participants ranges from 21-78 years. Mean age of

study participants was 41.95 ± 10**.** Mean age with standard deviation (SD) in males was 42 ± 10 years while in case of females it was 41 ± 10 years.

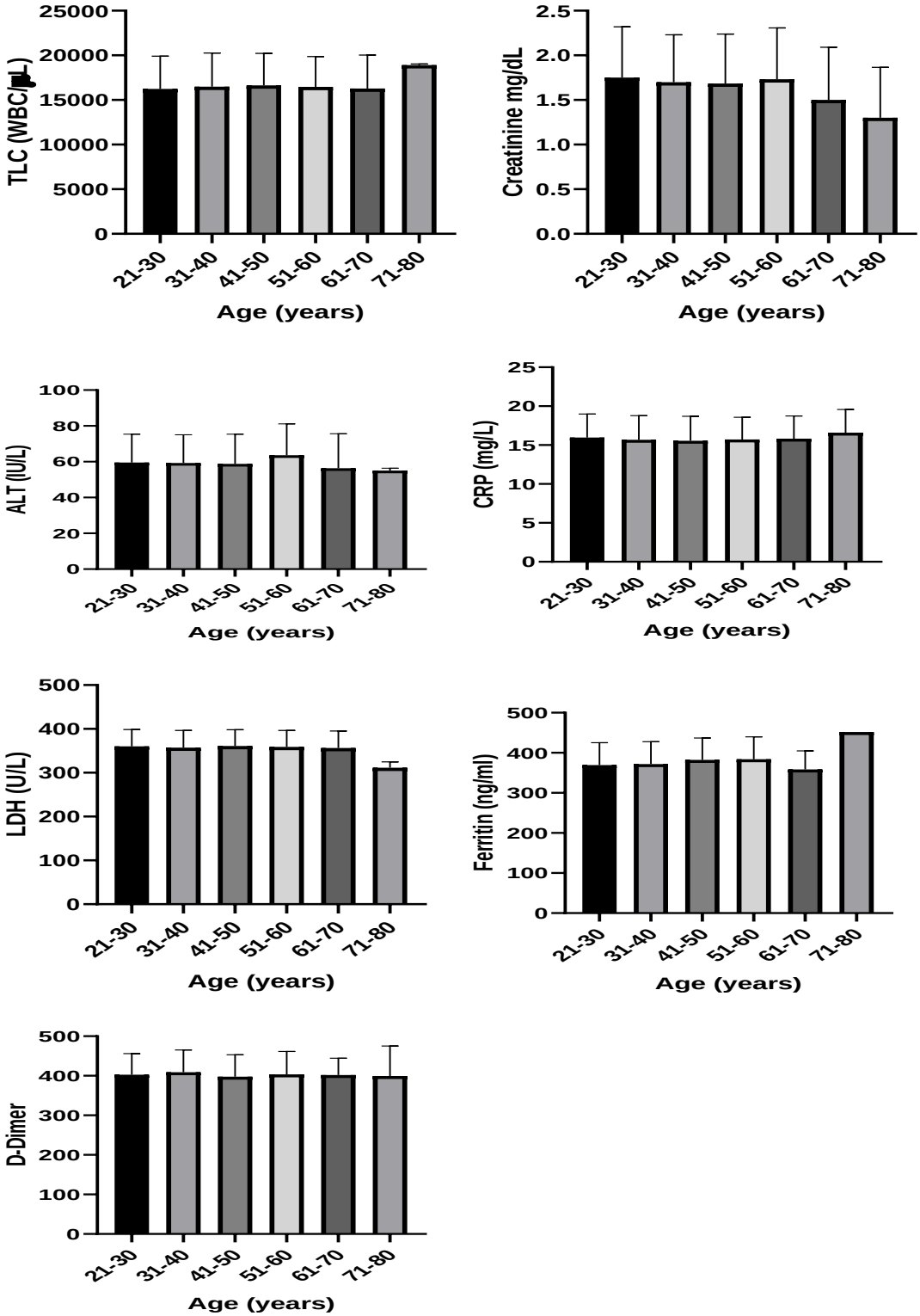

Fig.2. Laboratory parameters in different age groups suffering from COVID-19.

Laboratory parameters to check COVID-19 infection severity in different age groups

Different laboratory parameters including TLC, creatinine, ALT, CRP, LDH, Ferritin and D-dimer were analyzed for different age groups. Among all participants, 101 (17.03%) participants belonged to age group 21-30 years. 187 (31.53%), 199 (33.56%), 89 (15%), 15 (2.52%) and 2 (0.34%)participants were in age groups 31-40, 41-50, 51-60, 61-70 and 71-80 years, respectively. There was no statistically significant difference in TLC values among different age groups (P value 0.80). No significant difference was observed in case of creatinine (P value 0.51), ALT (P value 0.25), CRP (P value 0.91) and LDH (P value 0.50). There was a statistically significant difference (P value 0.03) in ferritin value of different age groups. While no significant difference was observed in mean values of D-dimer in age groups. Fig.2 depicts the laboratory parameters in different age groups suffering from COVID-19 and typhoid.

Laboratory parameters to check COVID-19 infection severity in males

Total leucocytes count (TLC) of male participants ranged from 11000-25200 cells/µL. Creatinine ranges from 0.8-3.0 mg/dL. ALT, CRP, LDH, Ferritin and D-Dimer ranges from 35-98 IU/L, 10.4-23.1 mg/L, 300-458 U/L, 288-458 ng/mL and 236-556, respectively. Laboratory parameters observed in male participants are presented in Table. 1.

Laboratory parameters to check COVID-19 infection severity in females

Total leucocytes count (TLC) of male participant's ranges from 11000-28000 cells/µL. Creatinine ranges from 0.8-2.8 mg/dL. ALT, CRP, LDH, Ferritin and D-Dimer ranges from 35-98 IU/L, 10.4-20.4 mg/L, 300-458 U/L, 288-458 ng/mL and 298-556, respectively. Laboratory parameters observed in female participants are presented in Table. 1.

Table1. Laboratory parameters in male and female participants

Parameters	Males (n=381)	Females (n=212)	P value
TLC	16572±3518	16349±3811	0.4719
Creatinine	1.62±0.57	1.83±0.4	<0.0001
ALT	59±16	60±17	0.7803
CRP	15.5±3.0	15.98±2.9	0.0875
LDH	361±38	353±36	0.0145
Ferritin	380±55	370±55	0.0344
D-Dimer	403±54	401±56	0.7220

DISCUSSION

COVID-19 prevalence is high in two highly populated provinces of Pakistan- Punjab and Sindh. Punjab is a most populated province of Pakistan consisting of 36 districts and 93 tehsils. According to National command and operation center (NCOC) first wave started from26 February, 2020 and second wave started from 28 October, 2020. During the second wave, mortality rate was higher than first wave of COVID-19 pandemic (Rahim *et al.,* 2022).

According to census conducted in 2017, population of Muzaffargarh was 4,322,009. The healthcare facilities however are limited in District Muzaffargarh. A study conducted to calculate the prevalence of Salmonella species indicate that in Muzaffargarh, prevalence of *S.typhi* is 10% (Qamar *et al.,* 2020). Moreover, Muzaffargarh was indicated as COVID-19 hotspot during second wave of pandemic. So, in the present study we aim to evaluate the cross antigenicity of COVID-19 and Typhoid in patients reported in Muzaffargarh.

Among the 593 patients,64% were males while 36% were females. Another study conducted in Attock stated that male population was more affected with COVID-19 as compared to female population (Ejaz *et al*., 2021). Mean age of participants was 41.95±10 years. It was observed that TLC, creatinine, ALT, CRP, LDH and D-dimer values were higher than normal in study participants. These results were consistent with another research conducted by Khan and co-researchers that represent the increase in these laboratory biomarkers in COVID-19 patients (Khan *et al.,* 2021). Laboratory parameters of participants that are represented in Fig 2. indicates that mean values of TLC, creatinine, ALT, LDH and D-dimer were same in all age groups. While there was significant difference in ferritin value was observed in different age groups. These results indicated that the age is not a factor to calculate the disease severity in patients suffering from COVID-19 as patients of all ages was equally affected.

Laboratory parameters were also compared among two groups-males and females, to check the infection severity. Statistically significant difference was observed in creatinine, LDH and ferritin levels in male and female

patients. Mean of creatinine level in female patients was higher than male patients while LDH and ferritin levels were higher in males. These results were not consistent with another research conducted in South Korea which represents that creatinine values were higher in male patients as compared to female patients (Kim and Sung, 2021). Another study indicated that creatinine levels increases in case of typhoid infection both in males and females (Ozougwu *et al.*, 2019). It was observed in a study that male patients suffering from COVID have high level of LDH (Ashraf *et al.*, 2022). LDH levels also increases in case of typhoid fever as reported by Sameera*et al.*(2013). Moreover, a significant difference in ferritin level in male and female COVID patients was observed in a recent study (Gandini *et al.*, 2021). TLC, ALT, CRP and D-Dimer values were also above the normal values among all the patients. Higher TLC, ALT and CRP was observed in case of COVID-19 during different studies (Anurag, Jha, and Kumar, 2020; Chen *et al*., 2021). Higher level of D-Dimers is known for its association with COVID-19 severity as reported by Yu and co-researchers (Yu *et al.,* 2020). However, in the present study, no significant difference in above mentioned markers was observed in different age groups or in different genders.

CONCLUSION

In the present study, it was observed that male population was more affected. Different age groups do not exhibit difference in all laboratory biomarkers except ferritin. Significant difference was observed in creatinine, LDH and ferritin levels in male and female patients. It can be concluded that all age groups are under same risk. However, disease severity is higher in male population.

Author's Contribution. All authors contributed equally.

Conflict of interest. There exists no conflict of interest for publishing this manuscript.

REFERENCES

Ahmad, S., C. Tsagkaris, A.T. Aborode, M.T.U. Haque, S.I. Khan, U.A. Khawaja, A.C. dos Santos Costa, M.Y. Essar and D.E. Lucero-Prisno III (2021). A skeleton in the closet: the implications of COVID-19 on XDR strain of typhoid in Pakistan. *Public Health in Practice,* 2:100084.

Ali, A., (2020). Second wave of Covid-19 in Pakistan; Are more episodes down the road? 2020.

Anurag, A., P.K. Jha and A. Kumar (2020). Differential white blood cell count in the COVID-19: A cross-sectional study of 148 patients. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews,* 14(6): 2099-2102.

Ashraf, A., A. Liaquat, S. Shabbir, S.A. Bokhari, Z. Tariq, Z. Furrukh, A.A. Raja and M.J. Khan (2022). High level of lactate dehydrogenase and ischaemia–reperfusion injury regulate the multiple organ dysfunction in patients with COVID-19. *Postgraduate Medical Journal*.

Chen, F., W. Chen, J. Chen, D. Xu, W. Xie, X. Wang and Y. Xie (2021). Clinical features and risk factors of COVID-19-associated liver injury and function: A retrospective analysis of 830 cases. *Annals of Hepatology,* 21:100267.

Ejaz, R., M. Ashraf, S. Qadeer, M. Irfan, A. Azam, S. Butt and S. Bibi (2021). Gender-based incidence, recovery period, and mortality rate of COVID-19 among the population of district Attock, Pakistan. *Brazilian Journal of Biology*, 83:e249125.

Gandini, O., A. Criniti, M. Gagliardi, L. Ballesio, S. Giglio, A. Balena, A. Caputi, A. Angeloni and C. Lubrano (2021). Sex-disaggregated data confirm serum ferritin as an independent predictor of disease severity both in male and female COVID-19 patients. *Journal of Infection,* 82(3):414-451.

Khan, M., N. Shah, H. Mushtaq and V. Jehanzeb (2021). Profiling laboratory biomarkers associated with COVID-19 disease progression: a single-center experience. *International Journal of Microbiology,* 2021.

Kim, S.-G. and H.H. Sung (2021). Status of Kidney Function in Hospitalised COVID-19 Patients in the Southern Gyeonggi Province, South Korea. *Korean Journal of Clinical Laboratory Science,* 53(3): 208-216.

Ozougwu, J., K. Alozie, C. Imakwu, S. Eziuzor and D. Akwari (2019). Changes in Renal Parameters Associated with Typhoid Infection. *Changes,* 38(2): 1-6.

Pascarella, G., A. Strumia, C. Piliego, F. Bruno, R. Del Buono, F. Costa, S. Scarlata and F.E Agrò (2020). COVID-19 diagnosis and management: a comprehensive review. *Journal of Internal medicine,* 288(2): 192-206.

Qamar, A., T. Ismail, and S. Akhtar (2020). Prevalence and antibiotic resistance of Salmonella spp. in South Punjab-Pakistan. *Plos one,* 15(11): e0232382.

Rahim, S., M. Dhrolia, R. Qureshi, K. Nasir and A. Ahmad (2022). A Comparative Study of the First and Second Waves of COVID-19 in Hemodialysis Patients From Pakistan. *Cureus,* 14(1).

Saeed, U., K. Sherdil, U. Ashraf, I. Younas, H. Butt and S. Ahmad (2021). Identification of potential lockdown areas during COVID-19 transmission in Punjab, Pakistan. *Public health*, 190:42-51.

Sameera, K., T. Sunitha and N. Kumar (2013). Evaluation of serum lactate dehydrogenase levels in typhoid fever *International journal of pharma and bio sciences*, 4:B944-B950

Yu, H.-H., C. Qin, M. Chen, W. Wang and D.-S. Tian (2020). D-dimer level is associated with the severity of COVID-19. *Thrombosis research*, 195: 219-225.

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