Open Access
Phytopathogenomics and Disease Control., 2023, 2(2):63-70
ISSN (Online):2957-5842; ISSN (Print):2957-5834
DOI: https://doi.org/10.22194/Pdc/2.1008
https://societyfia.org/journal/PDC
Society for Innovative Agiculture

Evaluation of Temperate Maize Inbred Lines for Iron (Fe) Concentration using Atomic Absorption Spectrophotometry

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Maize (*Zea mays* L.) is one of the important cereal crops, ranking third after wheat and rice. It holds the title of the "Queen" of cereals due to its wide cultivation and nutritional value it provides. Especially iron is crucial for the proper functioning of haemoglobin and blood formation. Unfortunately, insufficient consumption of iron-rich foods is the primary cause of iron deficiency in the underdeveloped countries. A research study was conducted at the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, aiming to investigate the diversity of 20 indigenous maize (*Zea mays* L.) inbred lines. Seeds were sown in a Randomized Complete Block Design (RCBD) with three replications. The study was conducted under open field conditions, focusing on genetic variations related to quality and yield traits. The results were analysed using analysis of variance (ANOVA) to determine variability among the inbred lines. Various parameters were recorded at maturity and after harvesting i.e., plant height, ear traits, and grain iron content. The evaluation of grain iron content revealed significant variation among the inbred lines tested. The inbred line with the highest iron content was 6162, recording 40.083 µg/g. Additionally, the analysis of other morphological and yield traits showed significant variations among the inbred lines. Three lines showed promising iron content: 6162 (40.083 µg/g), CRT-3 (36.417 µg/g), and 6199 (35.25 µg/g). Based on these research findings, there is a clear need to develop a breeding plan to enhance iron content in local maize germplasm.
Keywords: Maize, Iron deficiency, Field conditions, Diversity, Grain iron, Breeding plan.

INTRODUCTION

Maize (*Zea mays* L.) is third important cereal crop after wheat and rice (FAO, 2021), and often said to be as "Queen" of cereals. It is widely grown throughout the world for its edible grains (Duvick, 2005). It is member of Poaceae family and genus *Zea*, which also includes other important cereals like sorghum and teosinte (Hufford *et al.*, 2011). The diploid number of chromosomes of maize are 2n = 2x = 20, having a large genome size of 2.5 million base pairs (Schnable *et al.*, 2009). Maize plants are characterized by their tall, grass-like appearance, with narrow and long leaves and prominent tassel at the top of plant (Chandler and Brendel, 2002). It is a monoecious plant, having separate male and female flowers on the same plant i.e., pollens grow in tassel and ovary develop in ear respectively while ear specialized structure for grain development (Lrish and Nelson', 1989). Maize is an outcrossing crop, depending on wind for pollination but sometimes insects also play a role in pollination (Stuber *et al.*, 1992). This is the reason why maize is genetically highly diverse crop, having a wide range of variation in characters such as yield, grain quality, and resistance to pests and diseases. Its grains can have a variety of colours, including yellow, white, red, and blue. The colour of grain is mainly determined by the presence of several pigments, such as carotenoids and anthocyanins. These pigmentation agents not only contribute towards the development of colour of grain, but also have health benefits for humans and animals that has maize grains as part of their food and feed (Harjes *et al.*, 2008). Maize is believed to be originated from Mesoamerica, which includes parts of Mexico and Central America. Domestication of maize crop dates to 8000 years by indigenous peoples (Matsuoka *et al.*, 2002). At present, maize is grown worldwide, with the United States, China and Brazil

Karim, A., W.A. Karim, M. Umar, A. Mughal, H. Ghaffar, M.S. Nawaz, L. Irfan, M. Zafar, Z. Ali, M. Aslam and A. Ammar. 2023. Evaluation of Temperate Maize Inbred Lines for Iron (Fe) Concentration Using Atomic Absorption Spectrophotometry. Phytopathogenomics and Disease Control 2:63-70.
[Received 2 Sep 2023; Accepted 20 Nov 2023; Published 15 Dec 2023]

being the top three producers (FAO, 2020). It is the most important crop worldwide, in terms both economic value and its role as food for humans as well as animal feed. Among all the cereals there is an estimation of maize worldwide production about 1 billion metric tons per year. The genetic regulation of maize starch composition results in great diversity in the amylose to amylopectin ratio, making it ideal for a variety of industrial uses. Maize is also a great oil source, with a proven ability to lower blood cholesterol levels when taken by people. Maize has been used in a variety of ways across the world, resulting in the manufacture of several value-added products and fermented delicacies. Furthermore, maize naturally includes carotenoids such as beta-carotene, zeaxanthin, lutein, and cryptoxanthin, which have a variety of health advantages such as maintaining normal vision and reducing oxidative stress (Chaudhary *et al.*, 2014). Top corn producing countries include China, Brazil, United States of America, and Argentina (FAO, 2021). According to Pakistan Bureau of Statistics, Pakistan produces 6.6 million metric tons of corn from an area of approximately 1.1 million hectares. In Pakistan, maize is mostly cultivated in Punjab and Khyber Pakhtunkhwa provinces, Punjab is the major contributor to the country's total maize production. 0.5 % of gross domestic production of Pakistan is contributed by maize (GOP, 2022). For small scale farmers it is very important crop in Pakistan, specifically the areas where rain is the only sources of irrigation as it is the source of food feed and income. Nevertheless, maize production in Pakistan is facing a lot of challenges such as low yield, insufficient research and development, credit, and input access limitations as well as climate change-induced variability in rainfall patterns. Among all the mineral nutrients which are necessary in trace amounts for overall well-being of human beings as well as animals, Iron plays a paramount role in numerous metabolic events i.e., photosynthesis, respiration, chlorophyll synthesis, various redox reactions etc. (Briat and Lobréaux, 1997; Welch and Graham, 2004). Iron is a vital constituent of myoglobin and haemoglobin, playing their role in the supply and storage of oxygen. Most frequent dietary illness is anaemia, resulting from food that is iron deficient and troubling 32.9% of the world's population, with maximum risk of occurrence in southern Asia and sub-Saharan Africa (Wessells *et al.*, 2012; Kassebaum *et al.*, 2014). In females, during puberty as well as pregnancy, the highest iron deficiency effects (anaemia) can be seen. In the same way, iron deficiency disturbs cognitive growth of children until puberty. It also enhances the risk of getting infectious diseases as well as increased rate of mortality in children (Oliver and Gregory, 2015). Maize is a paramount constituent of various industrial products as well as animal feed (Nuss and Tanumihardjo, 2010), providing not only significant amounts of protein and energy, but also micronutrients for instance Zinc, Copper, and Iron (Shiferaw *et al.*, 2011; Dunn *et al.*, 2014). Iron (Fe) content in endosperm of grain of various maize genotypes may fluctuate from 144 to 168 µg/g (Bityutskii *et al.*, 2001). Iron transporter genes are essential in this process since they oversee iron uptake, transport, and distribution within the plant (Curie and Briat, 2003). Iron transporter genes in maize have been found and characterized, including ZmYS1, ZmIRT1, ZmIRT2, and ZmFRO1 (Vigani *et al.*, 2013). These genes have been found to play critical roles in iron absorption and transport in several maize tissues (Schaaf *et al.*, 2004), but ZmIRT2 may strictly work together with ZmIRT1 uptake Fe in roots. In 21st century, worldwide great efforts have been made about the potential to produce food of greater nutritious value because cereal crops are genetically deprived of basic micronutrients (White and Broadley, 2009). Hence, there is an urgent requirement to cope this burning issue of micronutrient deficiencies that is attributable to what is referred to as "Hidden Hunger" and is affecting no less than 2 billion people, most of them living in Latin America, southern Asia as well as sub-Saharan Africa (McGuire, 2015). Fortification of food has an extensive account of use in developed world, it depends on the supplementation of micronutrients to the processed foods. However, process of food fortification is likely to have very prompt but less sustainable effects, because of several cost, safety, and technological concerns may prove to be limiting such intrusions (Allen *et al.*, 2006). Moreover, it is impossible to provide this kind of intervention to the target populations (Pfeiffer and McClafferty, 2007). "Hidden Hunger" due to micronutrient deficient diets, is a major public health issue prevailing all over developing world including Pakistan. Among all the micronutrient deficiencies iron deficiency is most prevalent leading to anaemia and other health issues. Iron deficiency in Bangladesh is a major health issue specifically in women and children. A study showed that the prevalence of anaemia due to iron deficiency remained 51.9 % overall- 47.4 % in urban and 53.1 % in rural regions respectively (Khan *et al.*, 2016). To deal with this public health issue worldwide, many approaches are being employed. These approaches include agronomic fortification, biofortification, breeding, and genetic engineering. Agronomic fortification involves addition of iron fertilizers to the soil to increase the iron content in crops (Bhardwaj *et al.*, 2022), this method has been used successfully in many countries, including Pakistan. Biofortification is another approach which includes breeding staple crops for higher iron contents. This approach has been successful in increasing the iron contents in many crops, including maize, rice, and beans (Cakmak, 2008). Connorton and Balk, (2019) studied that breeding for increased iron uptake efficiency has been extraordinarily successful in increasing iron contents of different crops so far. Genetic engineering is also being explored as a potential approach to increase the iron contents of crops. This involves modification of genes responsible for iron transport and storage in plants. Although this approach is in its early stages hence it has shown very promising results in increasing the iron contents of rice and other crops (Lee *et al.*, 2009). Overall, a combination of all these approaches is likely to be the most effective way to address the iron deficiency and hidden hunger. These approaches can also have positive impacts on agricultural productivity and food security. By improving the amounts of micronutrients in the staple crops, micronutrient availability to the poor can be elevated, this will lead to the decrease in the predominance of micronutrient deficiencies. Plant breeding, as more sustainable approach, can be used to overcome the nutritional deficiencies, maize is a best food crop to reduce the prevalence of these nutritional deficiencies because it is one of the most affordable and easily available food crops Hence, research was conducted under the agro-climatic conditions of Faisalabad.

MATERIALS AND METHODS

The current research was conducted at the Maize Research Area of Department of Plant Breeding and Genetics at University of Agriculture, Faisalabad. Coordinates of experimental area are 31°26'26" N latitude and 73°04'14" E longitude. Total twenty (20) maize inbred lines 6193, 6162, 6267, 6071, 6159, 6255, 6060, 6154, 2075, 6199, 349-A, 6266, 6276, 6212, 6180, 31(4B), CH-131265, PBG-1(2), CRT-3 and 6161, were obtained from the seed bank of the Department of Plant Breeding and Genetics. These genotypes were subjected to testing for evaluation of grain iron content and their morphological characteristics. Soil samples from experimental field were evaluated for iron content and the value of iron was 29.2 µg/g. The germplasm was planted in the mid of February 2022. The RH% in February, March, April, and May were 82.9%, 66.4%, 42.5%, and 43.4% respectively. The average temperatures for these months were 16.3, 25.2, 31.5, and 34.7 °C respectively. The experimental design used for this investigation was Randomised Complete Block Design (RCBD) with three (3) replications. P×P and R×R spaces were set at 20 cm and 75 cm, respectively. The inbred lines were assigned to the experimental units using a random number table. Total 12 to 14 irrigation cycles were done based on the moisture levels in the field and the needs of the crop. Fertilizer applications were made in accordance with crop demand. Plants were also self-pollinated when reached the appropriate stage.

***preparation of samples for iron analysis*:**

***Grinding*:** When the ears were ready, they were harvested from plants separately, then dried in a clean, shaded area to lower the moisture percentage of the harvested grains to 14%. To get representative samples of the grains, they were thoroughly ground into powder using a Cyclotech Sample Mill, which confirmed that there was no iron contamination.

Digestion: Ground material was digested using a 7:3 combinations of two acids (HNO_3: $HClO_4$). The first cycle lasted sixty minutes at a temperature of 150 °C, followed by a thirty-five-minute cycle at a temperature of 235 °C.

Determination of iron (Fe) concentration through atomic absorption spectrophotometer: Minerals, especially iron (Fe), were measured in the prepared samples using an Atomic Absorption Spectrophotometer "Hitachi Polarised Zeeman AAS, Z-8200, Japan", as defined in AOAC, (1990). The instrument's operational parameters for these components are summarised in table (see Table 1).

***Standards of preparation*:** Calibrated standards were generated by mixing an aqueous solution (1000 ppm) with a commercially accessible standard solution (Applichem®). The advancement of working standards necessitated the use of highly purified de-ionized water. All glass equipment used in the analysis were submerged in 8N HNO_3 for a long period of time before being cleaned with several changes of de-ionized water to ensure the best degree of precision.

Table 1. Conditions used to Determine Iron (Fe) by Atomic Absorption Spectrophotometer.

Factors	Values for Fe
Wavelength (nm)	248.3
Slit Width (nm)	0.2
Lamp Current (mA)	10.0
Burner Head	Standard type
Flame	Air-C_2H_2
Burner Height (mm)	7.5
Oxidant gas pressure (Flow rate) (kpa)	160
Fuel gas pressure (Flow rate) (kpa)	6

RESULTS

***Plant height*:** Analysis of variance expressed significant differences in plant height among the inbred lines (Table 2). Further investigation using mean values showed the range of plant heights which varied from 85.83 cm to 162.33 cm. Inbred line 6255 exhibited the highest plant height at 162.33 cm, while genotype CRT-3(6210) displayed the lowest 85.83 cm (Figure 1). Similar results were also stated by Nzuve et al. (2014).

***Ear height*:** Analysis of variance expressed significant differences in ear height among the inbred lines (Table 2). Further investigation using mean values showed the range of ear heights which varied from 61.43 cm to 99.2 cm. Inbred line 6255 exhibited the highest plant height at 99.2 cm, while inbred line 6212 (CRT-3) displayed the lowest 61.43 cm (Figure 1). Similar results were also stated by Sherry *et al.* (2009).

***Ear length*:** Analysis of variance expressed significant differences in ear length among the inbred lines (Table 2). Further investigation using mean values showed the range of ear length which varied from 6.03 cm to 15.3 cm. Inbred line 349 (A) exhibited the longest ear of 15.3 cm, while inbred line

Table 2. ANOVA of Various Traits.

Source	PH	EH	EL	ED	EW	GPR	HGWt	FeC
Rep (MS)	238.90	144.39	2.16	0.06	182.67	1.80	5.73	1.53
Inbred (MS)	1362.50	271.04	23.28	1.50	927.30	116.80	18.49	348.62
Error (MS)	91.78	60.22	1.00	0.31	138.17	9.92	7.05	2.33
F-value	14.85**	4.50**	23.18**	4.83**	6.71**	11.77**	2.62*	149.80**
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000
CV%	7.67	10.46	9.04	12.07	10.27	11.62	13.45	7.71

6161 was recorded 6.03 cm long (Figure 1). Similar results were also stated by Ojo et al. (2007).

***Ear diameter*:** Analysis of variance expressed significant differences in ear diameter among the inbred lines (Table 2). Further investigation using mean values showed the range of ear diameter which varied from 3.23 cm to 5.93 cm. Inbred line 6266 exhibited the highest ear diameter of 5.93 cm, while inbred line 6161 was recorded least diameter of 3.23 cm (Figure 1). Similar results were also stated by Keskin *et al.* (2005)

***Ear weight*:** Analysis of variance expressed significant differences in ear weight among the inbred lines (Table 2). Further investigation using mean values showed the range of ear weight which varied from 71.33 g to 144.67 g. Inbred line 6180 exhibited the highest ear weight of 144.67 g, while inbred line 6159 recorded the least ear weight of 71.33 g (Figure 1). Similar results were also stated by Sherry *et al.* (2009).

***Number of grains per row*:** Analysis of variance expressed significant differences in number of grains per row among the inbred lines (Table 2). Further investigation using mean values showed the range of number of grains per row which varied from 14.67 to 43.67 grains. Inbred line 6060 exhibited the highest number of grains per row 43.67, while inbred line 6161 recorded the least number of grains per row 14.67 (Figure 1). Similar results were also stated by Ali *et al.* (2014); Zare *et al.* (2011).

***100 grain weight (g)*:** Analysis of variance expressed significant differences in 100 grain weight among the inbred lines (Table 2). Further investigation using mean values showed the range of 100 grain weight which varied from 15.63 g to 22.87 g. Inbred line 6266 exhibited the highest 100 grain weight of 22.87 g, while inbred line 6255 recorded the least 100 grain weight of 15.63 g (Figure 1). Similar results were also stated by Keskin *et al.* (2005)

***Grain iron (Fe) content (µg/g)*:** Analysis of variance expressed significant differences in grain iron content among the inbred lines (Table 2). Further investigation using mean values showed the range of grain iron content which varied from 6 to 40.083 µg/g. Inbred lines with the highest grain iron contents were 6162 40.083 µg/g, followed by CRT-3 36.417 µg/g, and the inbred line with the lowest grain iron content was 6212 6.00 µg/g. According to a recent study by Prasanna *et al.* (2011), a genotype HP2 (Harvest Plus) showed 42.21 µg/g. Similar results were also stated by Chakraborti *et al.* (2011), Phuke *et al.* (2017), Sharma *et al.* (2021), Qureshi *et al.* (2021), Saletnik *et al.* (2022) and Udo et al. (2023).

***Principle Component Analysis*:**

PC 1: PCA 1 accounts for a significant part of variability among inbred lines and traits which 31.3%. Traits like grains per row (GPR), hundred grain weight (HGWt), ear height (EH) and ear length (EL) contribute more to the variability captured by PCA 1. Inbred 6190 shows that it has trait values that are in positive association to ear length (EL) and hundred grain weight (HGWt). Inbred lines PBG1(2), 6255,6161, 6212, 6276, and 349-A are apart from one another showing that they are variable from one another and have district trait profiles.

PC 2: PCA 2 accounts for relatively moderate amount of variability among inbred line and all the traits which is 18.8%. PCA 2 is influenced by plant height (PH), ear weight (EWt), ear diameter (ED) and grain iron content (FeC). There is slight degree of association between ear diameter (ED) and ear weight (EWt) as they are positioned closer. Inbred Lines 6193 and 6154 are extremely close showing that they share similar trait profiles. Inbred lines CH-131265, 6199, 6266, 6162, and CRT-3 are more scattered and apart from one another indicating that they have different trait profiles.

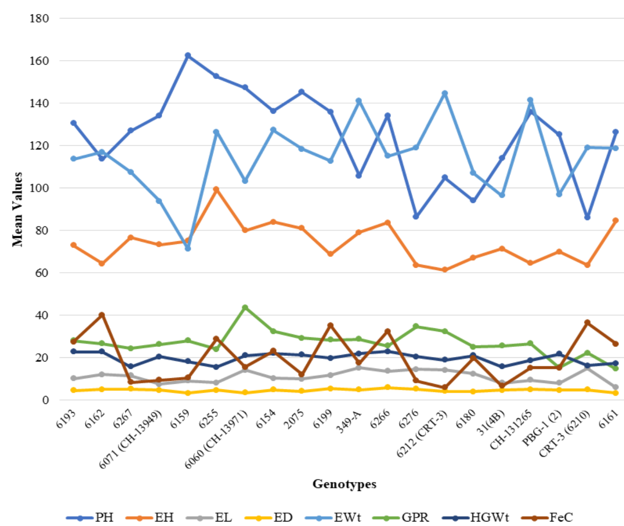

Figure 1. Mean values of various traits.

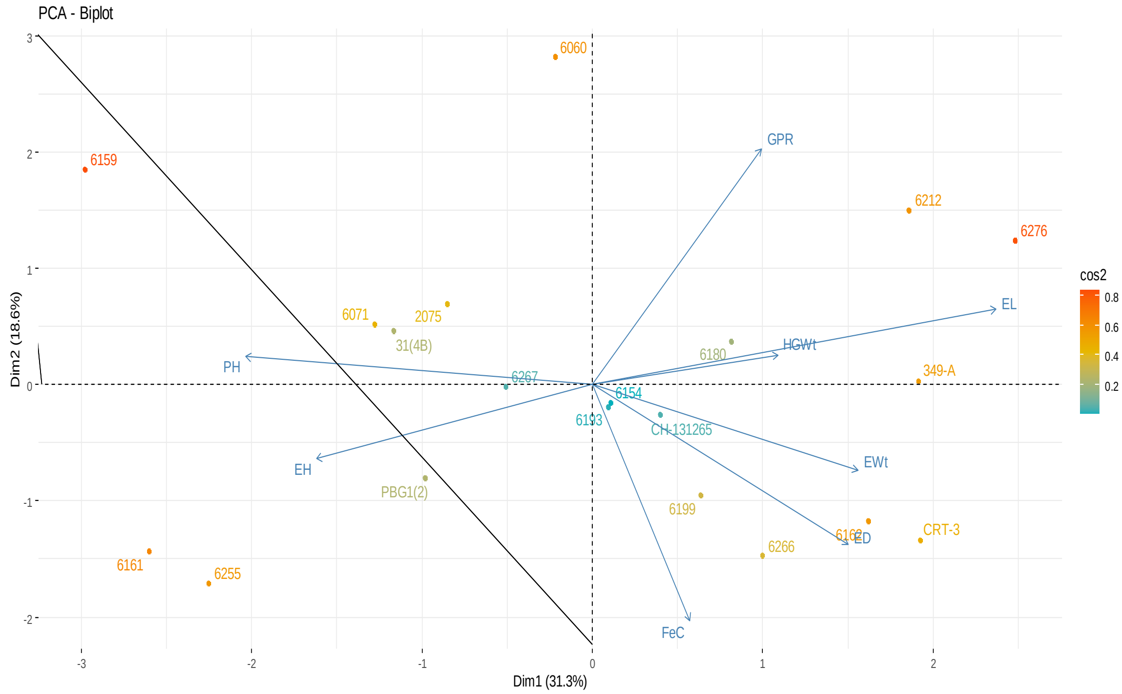

Figure 2. Principle Component Analysis - Biplot

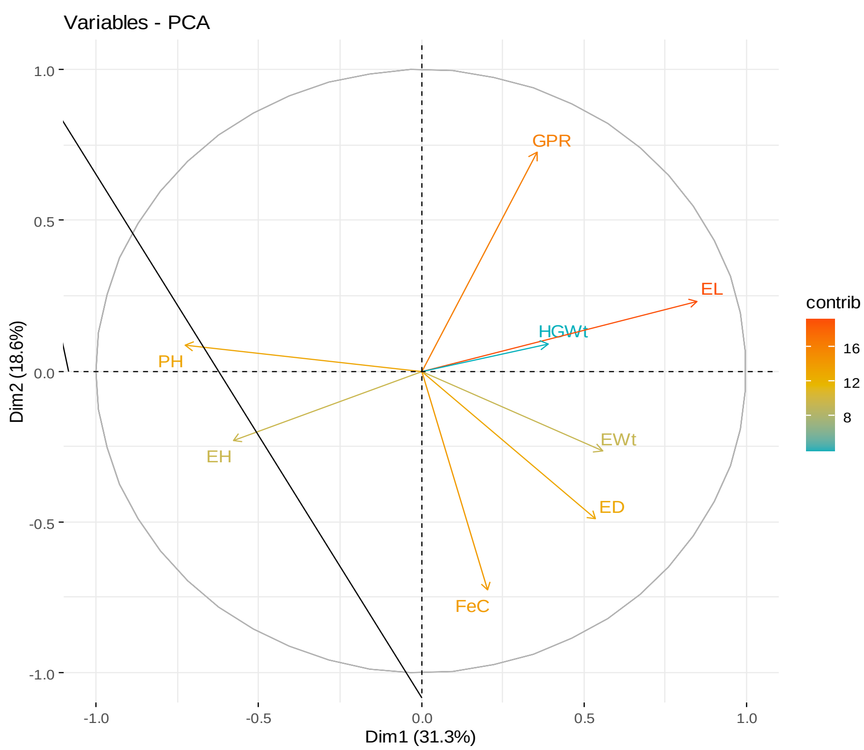

Figure 3. Variables – Principal Component Analysis

***Conclusion*:** The outcomes of our investigation reveal notable iron concentrations in three distinct lines: 6162 (40.083 µg/g), CRT-3 (36.417 µg/g), and 6199 (35.25 µg/g). These findings underscore the potential for targeted breeding initiatives aimed at augmenting the iron content within indigenous maize germplasm. The imperative to formulate a comprehensive breeding strategy is evident, reflecting the significance of advancing nutritional traits in maize for broader agricultural and public health objectives.

Authors' contributions**:** Abdul Karim performed this research. Muhammad Aslam supervised this research. Remaining authors equally contribute in formatting, editing, revision and proof reading of this article

Funding**:** NA

Ethical statement**:** This article does not contain any studies with human participants or animal performed by any of the authors.

Availability of data and material**:** NA

Code Availability: NA

Consent to participate**:** All authors are participating in this research study.

Consent for publication**:** All authors have no objection in publication of this research paper.

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