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**Abstract: Background:** Increased energy consumption in agriculture has led to growing environmental concerns and higher costs. The evolution of herbicide resistance in weeds may affect the herbicide application rate, energy consumption and greenhouse gas emissions. **Objective:** This study investigated the effect of herbicide resistance in weeds on energy consumption and greenhouse gas emissions in wheat fields of Golestan province, Iran. **Methods:** The data were collected from 351 wheat fields in Golestan province with respect to the proper distribution of sampling points. The farms were categorized into four classes according to the previous studies: 1) non-resistant fields; 2) fields with resistance to ACCase (acetyl-coenzyme A carboxylase) inhibitors; 3) fields with resistance to ALS (acetolactate synthase) inhibitors; and 4) fields with resistance to both ACCase and ALS inhibitors. Then,

**Keywords**: ACCase, ALS, CO<sub>2</sub>, inputs, weed

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**1. Introduction** 

Global population continues to grow; therefore, ensuring food security becomes increasingly vital. Consequently, agricultural production demands higher energy consumption. Energy sources are limited; on the other hand, excessive consumption of energy sources will have detrimental effects on the environment (Zhang et al., 2023). The agriculture section consumes energy directly, including fossil fuels or electricity for the machinery and farm equipment, or indirectly to produce chemical fertilizers, manufacturing of machinery and pesticides. Moreover, agricultural practices such as seedbed preparation, sowing, harvest, water pumping systems, etc. require energy (Soltani et al., 2013).

input and output energy, energy use efficiency, energy productivity, specific energy, net energy, percentage of direct, indirect, renewable and non-renewable energies, and greenhouse gas emission during wheat production were calculated. **Results:** Compared to non-resistant fields, evolution of resistance to herbicides led to increased energy consumption (5.7-8.2%) and yield loss (21.8-25.6%) and consequently, there was a decrease in net energy (25.6- 31.3%), energy productivity (27.2-31.8%) and energy use efficiency (24.7-30.4%). Also, greenhouse gas emissions from the non-resistant fields were 7.4-10.0% lower than in the fields with herbicide-resistant weeds. **Conclusions:** Noting the negative effect of herbicide resistance in terms of energy consumption and greenhouse gas emission, efforts to properly manage herbicide-resistant weeds are essential both economically and ecologically.

Increased consumption of resources in agriculture has led to growing environmental concerns such as high consumption of non-renewable energy sources, decreased biodiversity, water pollution by agrochemicals and adverse effects of pesticides (Carlson et al., 2017). The emission of greenhouse gasses such as carbon dioxide (CO<sub>2</sub>), dinitrogen oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>) to the atmosphere is among the inevitable consequences of excessive energy consumption, and agricultural activities constitute a significant part of this emission (Yi et al., 2024).

Energy sources in agriculture are categorized into natural (including solar energy and the energy stored in soil) and auxiliary (Kazemi et al., 2016). Auxiliary energy sources may be classified into direct and indirect, renewable and non-renewable. Direct energies include human labor and fuel, whereas indirect energies consist of the energy derived from seeds, fertilizers, pesticides and machinery. Also, fuel, pesticides, fertilizers and machinery are regarded as non-renewable energy sources, whereas the energy embodied in human labor and seeds is classified as renewable (Nandan et al., 2021).

Numerous researchers have studied energy consumption in agriculture. Soltani et al. (2014) reported that nitrogen fertilizer and fuel required for machinery had the highest share of energy consumption in canola fields of Golestan province, Iran, and good crop management practices can reduce the application of nitrogen fertilizer and total energy consumption by 17 and 25%, respectively. Furthermore, good crop

management in canola increased output energy by 35%. Another study on rainfed canola revealed that nitrogen fertilizer, diesel fuel and machinery constituted 44, 30 and 14% of greenhouse gas emissions (Kazemi et al., 2016). A study comparing different soybean production methods in Iran found that the conventional scenario (most operations done using less powerful tractors or manually) had the highest energy efficiency at 3.18 kg MJ<sup>-1</sup>. In contrast, the mechanized scenario (use of modern equipment and high consumption of chemicals) had lower water consumption. Additionally, greenhouse gas emissions were lower in the conventional scenario than in the mechanized one. Electricity accounted for 78% of energy consumption in the mechanized method, while fuel was the primary energy source in the conventional method at 37% (Alimagham et al., 2017). In another study on wheat, seedbed preparation, sowing and nitrogen fertilizers had the highest share of energy consumption, and good farming practices can reduce energy consumption and greenhouse gas emissions by 11 and 20%, respectively (Soltani et al., 2013).

As mentioned, pesticides including fungicides, insecticides and herbicides are among the inputs contributing to energy consumption and greenhouse gas emissions in agriculture. Herbicides have enabled the growers to control the weeds efficiently and rapidly, improving crop production. However, over-reliance on these agrochemicals has resulted in weed flora shift and the evolution of herbicide resistance in weeds (Gherekhloo et al., 2021). Herbicide-resistant weed species can survive the herbicides at rates that are normally lethal to wild-type species and complete their life cycle (Leon et al., 2024). Resistance to herbicides has been reported in 272 weed species (Heap, 2024). Among the species with resistance to herbicides in Iran, the following stand out: little seed canary grass (*Phalaris minor* Retz.) (Gherekhloo et al., 2020) wild mustard (*Sinapis arvensis* L.) (Gherekhloo et al., 2018); short-spiked canary grass (*Phalaris brachystachys* Link.) (Golmohammadzadeh et al., 2019), barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) (Haghnama, Mennan, 2020), winter wild oat (*Avena sterilis* subsp. *ludoviciana* (Durieu) Gillet & Magne) (Hassanpour-Bourkheili et al., 2021), etc.

Wheat, one of the most important crops in the world, as well as Golestan province and Iran (Soltani et al., 2013), has the most cases of herbicide-resistant weeds in the world among the major crops (Heap, 2024). Due to the yield loss inflicted by the herbicide-resistant weeds in case of inappropriate weed management, it may be hypothesized that the evolution of herbicide-resistant weeds decrease crop yield, increase frequencies and rates of herbicide applications, leading to reduced energy use efficiency, and elevate greenhouse gas emissions. However, no reports are available on this issue. Thus, the present study investigated the effect of herbicide resistance in weeds on energy use and greenhouse gas emission in wheat fields of Golestan province, Iran.

# **2. Material and Methods**

#### **2.1 Sampling and site description**

The present study was conducted in Golestan province (located between 36° 44' N, 38° 05' N and 53° 51' E, 56° 14' E) in the North of Iran. The data were collected during the 2018-2019 growing season from 351 wheat fields in Golestan province with respect to the proper distribution of sampling points. Efforts were made to select farms with comparable management practices and climatic conditions, aiming to represent the majority of farmers in the region as closely as possible. Management operations conducted in the sampled farms are presented in Table 1. The sampling points were selected randomly within the wheat fields of the province containing weed species susceptible or resistant to herbicides using Equation 1 (Kazemi et al., 2016).

$$
n = (\Sigma N_h S_h) / (N^2 D^2 + \Sigma N_h S_h^2)
$$
 (1)

in which n is the number of required samples, N is the total number of fields in the population,  $\mathrm{N}_\mathrm{h}$  is the number of the population in h,  $S<sup>2</sup><sub>h</sub>$  is the variance of h stratification,  $D^2 = d^2/z^2$ : d is precision, and z is reliability coefficient. The permissible error was set to be 5 percent at a confidence level of 95 percent. Therefore, the sample size was calculated as 351 farms.



It must be noted that the province has been monitored for herbicide resistance for more than a decade and therefore, the location of wheat fields with herbicide-resistant species was well known to the authors (Gherekhloo et al., 2018; 2021; Golmohammadzadeh et al., 2019; Hassanpour-

fields (55%); 2) fields with resistance to acetyl- coenzyme A carboxylase (ACCase) inhibitors (21%); 3) fields with resistance to acetolactate synthase (ALS) inhibitors (13%); and 4) fields with resistance to both ACCase and ALS inhibitors (11%). The data were collected from the wheat growers by conducting a face-to-face questionnaire and statistical yearbooks. Also, crop management practices in the fields were monitored and recorded. The data were then entered into Microsoft Excel v. 2013 software and analyzed.

# **2.2 Energy analysis**

All inputs and outputs were calculated per hectare. Also, the energy consumed by the machinery for farm operations was calculated based on the equipment weight, the time required to complete that operation, and the economic life of the machinery (Soltani et al., 2014). Energy inputs including human labor, diesel fuel, chemical fertilizers, machinery, herbicides, seeding rate, and output (yield) were used for energy analysis. Energy equivalent for each input and output (Table 2) was used for the calculation. The average value for each input (Table 3) was multiplied by its energy equivalent, and the total energy of that input was calculated. Also, indices of energy use efficiency, energy productivity, specific energy, and net energy were calculated using Equations 2-5 based on the total energy equivalent of inputs and outputs (Soltani et al., 2014). Since wheat grain yield constituted the major part of the grower's income in the province, the output related to wheat straw was not considered in calculating specific energy and energy productivity (Soltani et al., 2013). It must also be noted that the fields were rain-fed, and fungicides and insecticides had not been applied in the studied fields.

Energy use efficiency = 
$$
\frac{\text{Energy output (MJ ha-1)}}{\text{Energy input (MJ ha-1)}} (2)
$$

Energy productivity = 
$$
\frac{Grain yield (kg ha-1)}{Energy input (MJ ha-1)} (3)
$$

$$
Specific\ energy = \frac{Grain\ Energy\ input\ (MJ\ ha^{-1})}{yield\ (kg\ ha^{-1})} \tag{4}
$$

Net energy = Output energy  $(MJ ha^{-1})$  – Input energy  $(MJ ha^{-1})$  (5)

Similar letters in each row indicate non-significant difference based on the LSD test at p<0.05. Values in parentheses denote standard deviation

# **2.3 Greenhouse gas emission**

The emission of greenhouse gasses in wheat fields containing herbicide-resistant and susceptible weeds was calculated as kg CO<sub>2</sub> equivalent based on the quantity of each input. Coefficients related to CO<sub>2</sub> equivalent resulting from wheat production practices are presented in Table 2.

# **2.4 Statistical analysis**

The present study was considered a completely randomized design, with each studied field serving as a replicate. Treatments included four different herbicide resistance scenarios (no resistance (NR), resistance to ACCase inhibitors (R-ACCase), resistance to ALS inhibitors (R-ALS), and resistance to ACCase + ALS inhibitors (R-ACCase + R-ALS). Analysis of variance was done using the SAS v.9.0 software. The means were compared using the least significant difference (LSD) method at  $p < 0.05$ . Microsoft Excel v. 2013 was employed to prepare the figures.





Similar letters in each row indicate non-significant difference based on the LSD test at p<0.05. Values in parentheses denote standard deviation.

### **3. Results and Discussion**

## **3.1 Energy analysis**

The energy used by human labor increased as a result of herbicide resistance in weeds (Table 4). The total energy of this input was 394.4 MJ ha $^{-1}$  in NR fields, 422.4 MJ ha $^{-1}$  in R-ACCase fields, 427.5 MJ ha<sup>-1</sup> in R-ALS and 441.0 MJ ha<sup>-1</sup> in R-ACCase + R-ALS fields. Also, the energy related to diesel fuel had a similar trend and increased by 6.6, 8.2 and 11.2% as a result of resistance to ACCase, ALS, and ACCase + ALS inhibitors compared to NR fields.

Machinery used  $1,526.9$  MJ ha<sup>-1</sup> of energy in non-resistant fields, which increased by 20.4- 25.2% in fields with herbicideresistant weed species. The energy related to the herbicides used for weed control also increased significantly due to herbicide resistance. In NR fields, 574.1 MJ ha<sup>-1</sup> of energy was consumed by herbicides, whereas this value increased by 59.9, 70.0 and 94.9% due to resistance to ACCase, ALS, and ACCase + ALS inhibitors, respectively. The energy consumed by other inputs including nitrogen, phosphorous, and potassium fertilizers and seeding rate did not follow a certain trend in the studied farms and varied between 5,744.9-5,884.3, 731.5- 758.1, 235.8-251.3 and 2,253.0-2,300.5 MJ ha-1, respectively. Overall, the total energy used for wheat production was 16,061.0 MJ ha<sup>-1</sup> in NR fields, 16,974.7 MJ ha<sup>-1</sup> in R-ACCase, 17,123.5 MJ ha<sup>-1</sup> in R-ALS and 17,385.8 MJ ha<sup>-1</sup> in R-ACCase + R-ALS fields. The share of each wheat production input from the total energy is presented in Figure 1, with nitrogen fertilizer and fuel as the inputs with the highest share of energy consumption in all four categories. The share of herbicides from the total energy rose from 3.6% in NR fields to 5.4-6.4% in fields infected by herbicide-resistant weeds.

Herbicide resistance decreased the energy resulting from wheat grain and straw yield. The grain yield energy in NR fields was 52,361.4 MJ ha-1, whereas this value was calculated at 40,939.5 MJ ha<sup>-1</sup> in R-ACCase, 39,837.0 MJ ha<sup>-1</sup> in R-ALS and  $38,955.0$  MJ ha<sup>-1</sup> in R-ACCase + R-ALS fields. Also, the

energy embedded in wheat straw in NR, R-ACCase, R-ALS and R-ACCase + R-ALS fields were 43,660.0, 35,520.0, 34,086.3 and 33,300.0 MJ ha<sup>-1</sup>, respectively. Overall, total output energy in NR fields was  $96,021.4$  MJ  $ha^{-1}$ , which reduced by 20.3, 23.0 and 24.7% compared to NR fields in R-ACCase, R-ALS and R-ACCase + R-ALS fields, respectively.

Similar letters in each row indicate non-significant difference based on the LSD test at p<0.05. Values in parentheses denote standard deviation

As mentioned, more than 60% of the energy consumed during wheat production in all four categories was related to nitrogen fertilizer and fuel. Application of nitrogen fertilizer at high rates increases energy consumption and leads to environmental issues such as nitrogen leaching and the pollution of the nutrient cycle (Tamagno et al., 2022). A significant part of the fuel used during farm operations is consumed by tractors. Due to the temporal depreciation of the machinery in most farms in the Golestan province, fuel consumption and energy use will grow (Kazemi et al., 2016).

The energy used by fuel, machinery and herbicides in wheat fields containing herbicide-resistant weeds was higher than that of NR fields. One might interpret that this increase in the energy use by fuel and machinery was consumed to control the herbicide-resistant weeds that survived the herbicide application at the recommended field rate. Therefore, machinery had to work for longer durations than the NR fields and consumed more fuel as well due to the repeated herbicide application. Also, compared with R-ACCase fields, fuel and machinery energy consumption increased by 1.4 and 0.7% in R-ALS and 4.3 and 2.3% in R-ACCase + R-ALS fields, respectively. This may be due to the failure of dual-purpose herbicides that are common in the region, i.e. mesosulfuron methyl + iodosulfuron methyl to control ACCase- resistant weeds and further evolution of resistance in broadleaf weeds such as turnipweed (Hatami et al., 2016) and wild mustard (Gherekhloo et al., 2018)



Similar letters in each row indicate non-significant difference based on the LSD test at p<0.05. Values in parentheses denote standard deviation.



**Figure 1** - Share of inputs used for wheat production in each herbicide resistance category in Golestan province. a) Non-resistant fields; b) fields with resistance to ACCase inhibitors; c) fields with resistance to ALS inhibitors; d) fields with resistance to ACCase + ALS inhibitors

to tribenuron methyl herbicide. Currently, the herbicide families used in the region are not diverse; farmers only use ACCase and ALS herbicides. Therefore, the farmers either applied increased herbicide rates or repeated the spraying.

According to the results, grain and straw yields in NR fields were higher than those of the fields containing herbicide-resistant weed species. This indicates that the application of herbicides at higher rates or repetition of herbicide application had been unable to control herbicideresistant weeds and only led to increased herbicide application rate and energy consumption. In other words, the farmer must spend more on inputs while gaining less due to lower yields. This illustrates the ecological and economic importance of proper management of herbicideresistant weeds.

### **3.2 Energy forms and indices**

Energy use efficiency in NR fields was 5.9, and the evolution of wee resistance to herbicide decreased this value, so energy use efficiency in R-ACCase, R-ALS, and R-ACCase + R-ALS fields was 4.5, 4.3, and 4.1, respectively. Also, energy productivity in NR fields was 0.22 kg MJ<sup>-1</sup>, which decreased to 0.15-0.16 kg  $MJ<sup>-1</sup>$  in fields containing herbicide-resistant weeds. This trend was reciprocal for the specific energy. The net energy of wheat production in NR fields was calculated at 79,940.45 MJ ha<sup>-1</sup> which decreased by 25.6% in R-ACCase fields, 28.9% in R-ALS fields and 31.3% in R-ACCase + R-ALS fields. Direct energy, indirect energy, renewable energy, and non-renewable energy forms in the four studied categories were statistically similar and varied from 29.7-33.1, 66.8-77.2, 16.6-17.0 and 82.9- 83.3%, respectively, which was in accordance with the findings of Soltani et al. (2013) (Table 5).

Similar letters in each row indicate non-significant difference based on the LSD test at p<0.05. Values in parentheses denote standard deviation

The results indicate that the evolution of resistance to herbicides has led to significant changes between the NR fields and fields with herbicide- resistant weeds in terms of energy productivity, energy use efficiency, and net energy, which was due to the increased energy use as a result of more consumption of energy by fuel, machinery

and herbicides. Although no difference was observed between the NR fields and fields with herbicide-resistant weeds in terms of energy forms, the slight reduction in the percentage of non-renewable and indirect energies in R-ACCase, R-ALS, and R-ACCase + R-ALS fields may be due to increased machinery operation and fuel, which were in turn a consequence of higher herbicide application rate due to repeated application (up to four repetitions). Integrating all weed management approaches (biological, mechanical, chemical, and cultural) is essential to reduce the reliance on chemical weed control measures. This will mitigate the energy consumption imposed by chemical herbicides as a source of non-renewable energy, which is essential to fulfilling the goals of sustainable agriculture (Farooq, Pisante, 2019).

#### **3.3 Greenhouse gas emission**

The results related to the greenhouse gas emitted from the studied farms are presented in Table 6. Greenhouse gas emission by diesel fuels in NR fields was calculated at 341.9 kg CO $_{2}$  equivalent ha $^{\text{-}1}$ , which increased by 6.6-11.2% due to herbicide resistance. Also, machinery operation led to 191.0 kg CO $_{\textrm{\tiny{2}}}$  equivalent greenhouse gas emission per hectare in NR fields, whereas this value was increased by 22.4-25.2% in fields with herbicide-resistant weed species. Also, a greenhouse gas emission of 59.0 kg CO $_{\textrm{\tiny{2}}}$  equivalent ha<sup>-1</sup> was recorded in NR fields due to herbicide application to control the weeds. Compared to NR fields, this value was 60, 70, and 94.6% higher in R-ACCase, R-ALS, and R-ACCase + R-ALS fields, respectively. Greenhouse gas emission in other inputs was not significantly different among the four categories. Nitrogen, phosphorous and potassium fertilizers and seeds emitted 418.1-428.2, 60.00-62.2, 19.4-20.6, and 78.4-80.0 kg  $CO<sub>2</sub>$  equivalent ha<sup>-1</sup>, respectively. Overall, total greenhouse gas emission in NR, R-ACCase, R-ALS and R-ACCase + R-ALS fields was calculated at 1,181.5, 1,276.5, 1,290.7, and 1,313.50 kg CO $_{_2}$  equivalent ha $^{\text{-}1}$ , respectively.

Similar letters in each row indicate non-significant difference based on the LSD test at p<0.05. Values in parentheses denote standard deviation

The share of each input in greenhouse gas emission is presented in Figure 2, according to which nitrogen



Similar letters in each row indicate non-significant difference based on the LSD test at p<0.05. Values in parentheses denote standard deviation.



Similar letters in each row indicate non-significant difference based on the LSD test at p<0.05. Values in parentheses denote standard deviation.



**Figure 2** - Share of inputs in greenhouse gas emission during wheat production in each herbicide- resistance category in Golestan province a) Non-resistant fields; b) fields with resistance to ACCase inhibitors; c) fields with resistance to ALS inhibitors; d) fields with resistance to ACCase + ALS inhibitors

fertilizer and fuel ranked first and second in all four categories. The percentage of greenhouse gas emissions by herbicides increased due to herbicide resistance, which may be attributed to increased machinery operation, fuel consumption and herbicide application to control herbicide-resistant weeds. It must be noted that the increase in the share of herbicides in greenhouse gas emission was much higher than the other inputs, further indicating the detrimental effect of herbicide-resistant on the environment.

Increased emission of greenhouse gasses, which according to the present study's findings, may arise from herbicide resistance, can also negatively impact weed control. Both crops and weeds are prone to be affected by climate change (Korres et al., 2016) and changes in temperature, CO<sub>2</sub>, and precipitation changes may alter the plant phenology, metabolism rate, and yield. These changes may vary depending on the species and plant characteristics. For instance, competition between the crop and C3 and C4 weeds may change due to climate change, and alternative weed management programs to mitigate the yield loss may be inevitable (Vilà et al., 2021). Moreover, increased temperature and atmosphere CO<sub>2</sub> may decrease the efficacy of herbicides by altering herbicide translocation and metabolism and lead to the evolution of conditional resistance in weeds (Matzrafi et al., 2018).

## **3.4 Implications for management**

As mentioned, herbicide resistance in weeds will lead to adverse ecological and economic situations for crop growers. The introduction of herbicides allowed the growers to adopt conservation tillage practices with more confidence, as these agrochemicals eliminate weeds swiftly without disturbing the soil (Dentzman, Burke, 2021). Conservation tillage, in turn, will result in lower energy consumption by reducing the amount of fuel and machinery used. Moreover, tillage is considered one of the most important causes of  $\mathrm{CO}_2^{}$  emission from soil (Hussain et al., 2021). Therefore, reduced tillage frequency can improve soil organic matter and carbon sequestration (Chen et al., 2022). The evolution of herbicide resistance in weeds may severely reduce the willingness of growers to adopt conservative tillage, as the farmers may turn to extreme plowing to tackle herbicideresistant weeds where the chemicals fail (Beckie, Harker, 2017). This issue threatens the sustainability of agricultural production, and therefore, it is essential to devise weed management plans to tackle this problem.

According to the present study's findings, more herbicide was used in fields containing herbicide-resistant weeds than in NR fields. However, this increase did not mitigate the adverse effect of weeds on wheat yield, indicating the need for applying herbicides with different modes of action. Diversification of herbicide usage is a great tool to hinder the further development of resistance to herbicides in weeds (Harries et al., 2020). This issue is even more important for

herbicides with a high risk of resistance evolution, such as ACCase and ALS inhibitors (Moss et al., 2019), which are widely used in Golestan province. Therefore, it is necessary to alternate between high-risk and low-risk herbicides to delay the evolution of herbicide resistance (Beckie, Harker, 2017).

Although increased diversity of herbicides can be a good strategy, it may not be possible without crop rotation. In regions such as Golestan province, where the growers heavily rely on a limited number of herbicides- mostly ACCase and ALS inhibitors- consecutive cultivation of a crop such as wheat will not lead to an efficient herbicide rotation required to control herbicide-resistant weeds (Gherekhloo et al., 2018; 2021). Therefore, it is necessary to sow a different crop to allow the application of herbicides with different modes of action or to adopt mechanical weed control methods (Beckie, Harker, 2017). This way, the excess energy consumption and greenhouse gas emissions resulting from repeated herbicide application may be eliminated. It must be noted that some mechanical methods such as chisel and moldboard plowing consume more energy compared with herbicides (Coleman et al., 2019). However, the farmers can use the chemical method again in a few years once herbicide resistance issue is solved Furthermore, better weed control may lead to improved yield and thus, improved energy use efficiency and energy productivity.

### **4. Conclusions**

Herbicide resistance in wheat production in Golestan province led to increased energy consumption (5.7-8.2%), yield loss (21.8-25.6%), and consequently, reduced net energy (25.6- 31.3%), energy productivity (27.2-31.8%), and energy use efficiency (24.7-30.4%). Also, greenhouse gas emissions in the fields containing herbicide-resistant weed species was 8.0-11.1% higher than in the fields with non-resistant weeds. Due to these negative ecological and environmental impacts of herbicide resistance in weeds, efforts to devise proper weed management programs are critical to achieve sustainable agriculture.

# **Author's contributions**

All authors read and agreed to the published version of the manuscript. SH and JG: conceptualization of the manuscript and development of the methodology. KH, SS, AT, and MT: data collection and curation. SH: data analysis. SH, JG, AS, and HK: data interpretation. KH: funding acquisition and resources. JG: project administration. JG: supervision. SH: writing the original draft of the manuscript. JG, AS, HK, FZ, MMT, and RD: writing, review and editing.

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