Differential Diagnosis of Iron-Deficiency Anemia from β-Thalassemia Trait Using an Intelligent Model in Comparison with Discriminant Indexes

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ABSTRACT

Introduction: Iron deficiency anemia (IDA) and β-thalassemia trait (β-TT) are the most common types of microcytic hypochromic anemias. The similarity and the nature of anemia-related symptoms pose a foremost challenge for discriminating between IDA and β-TT. Currently, advances in technology have gave rise to computer-based decision-making systems. Therefore, advances in artificial intelligence have led to the emergence of intelligent systems and the development of tools that can assist physicians in the diagnosis and decision-making. Aim: The aim of the present study was to develop a neural network based model (Artificial Neural Network) for accurate and timely manner of differential diagnosis of IDA and β-TT in comparison with traditional methods. Methods: In this study, an artificial neural network (ANN) model as the first precise intelligent method was developed for differential diagnosis of IDA and β-TT. Data set was retrieved from Complete Blood Count (CBC) test factors of 268 individuals referred to Padad private clinical laboratory at Ahvaz, Iran in 2018. ANN models with different topologies were developed and CBC indices were examined for diagnosis of IDA and β-TT. The proposed model was simulated using MATLAB software package version 2018. The results showed the best network architecture based on the advanced multilayer algorithm (4 input factors, 70 neurons with acceptable sensitivity, specificity, and accuracy). Finally, the results obtained from ANN diagnostic model was compared to existing discriminating indexes. Results: The results of this model showed that the specificity, sensitivity, and accuracy of the proposed diagnostic system were 92.33%, 93.13%, and 92.5%, respectively. i.e. the model could diagnose frequent occurrence of IDA in patients with β-TT. Conclusion: The results and evaluation of the developed model showed that the proposed neural network model has a proper accuracy and generalizability based on the initial factors of CBC testing compared to existing methods. This model can replace the high-cost methods and discriminating indices to distinguish IDA from β-TT and assist in accurate and timely manner diagnosis.

Keywords: β-thalassemia trait, Iron deficiency anemia, Differentiate diagnosis, Neural network based model.

1. INTRODUCTION

Microcytic anemia is characterized as the presence of trifling, often hypochromic, red blood cells in a peripheral blood smear and is typically categorized by a low MCV (less than 83 micron 3). Iron-deficiency anemia is the most communal cause of microcytic anemia and globally is considered as the most common cause of anemia (1, 2).

Iron Deficiency Anemia (IDA) is the major public health problems in developing countries and contributes to fatigue and reduced work capacity in adults, as well as, it causes digestive, neurological, and immune system disorders. Furthermore, IDA affects cognitive and mental development in children and adolescents (2-4). There is evidence that teenage girls are predominantly susceptible to iron-deficiency anemia. Similarly, studies in adult women show a correlation between IDA and fatigue (5).

Additionally, IDA can worsen chronic kidney disease and congestive heart failure (4). Generally, IDA is classically designated as a microcytic anemia. Thalassemia trait (minor) is among the most common types of microcytic anemia (5).

Thalassemia is a genetic blood disorder, caused when the body makes fewer healthy red blood cells and less hemoglobin than normal. Hemo-
globin is a protein in the red blood cells that carries oxygen to all body tissues (6).

Hemoglobin normally consists of two α- and two β-globin chains. The nature of the thalassemia syndromes is associated with the reduced or absent synthesis of one of these two chains. The prevalence of α-thalassemia is lower than that of β-thalassemia and is usually asymptomatic (7).

Thalassemia is the most common single-gene disorder throughout the world and represents a major public health problem, especially are widespread throughout the Mediterranean region, the Middle East, Southeast Asia, and some parts of Africa. There are different types of thalassemia characterized by abnormal hemoglobin production, the most common are α-thalassemia and β-thalassemia (8).

Studies have shown that thalassemia and hemoglobinopathies are the most common single gene disorders in Iran (9). Thalassemia is more prevalent in Iran along the outskirts of Caspian Sea, Persian Gulf, and Oman Sea and the highest frequency is more common in several provinces, including Mazandaran, Gilan, Khuzestan, Fars, Bushehr, Hormozgan, as well as Sistan and Baluchestan (10).

Most of the current hemoglobinopathy screening methods include high performance liquid chromatography (HPLC), hemoglobin electrophoresis, screening of PCR mutations, and DNA tests. All of these methods yield a higher project cost and require specialized instrumentation and trained technicians (8).

As well as, currently various red blood cell (RBC) indices and formulas have been designed to differentiate between thalassemia and IDA and their sensitivity and specificity are presented in various articles. However, manual conventional methods provide calculation disadvantages (maximum validity of 90%, Mentzer formula). Furthermore, Other drawbacks of manual indices include the differences between classification criteria for patient’s selection checklist, diversity across variables (age, race, sex), and the difference in HbA2 and serum hemoglobin and ferritin levels which make it difficult to compare the results and can affect the focus and outcome of studies (10).

Considering the similarity of the symptoms of anemia, diagnosis of β-thalassemia trait (β-TT) from iron deficiency anemia is blurred. Therefore, differential diagnosis of β-thalassemia trait from iron deficiency anemia is significant. Laboratory methods are currently used for differential diagnosis which is too expensive and time consuming. The diagnosis of β-TT is performed by complete blood count (CBC) and Hemoglobin A2 (HbA2) tests or electrophoresis technique. The elevated HbA2 level (≥ 3.5%) confirms the diagnosis of β-thalassemia trait. Similarly, in some equipped laboratories comprehensive DNA analysis and Hb electrophoresis are used for diagnosis of β-thalassemia trait (7).

Diagnosis of iron deficiency anemia can be performed easily through the routine CBC test or iron and serum ferritin measurements (7). Sometimes, bone marrow biopsy analysis is required to make a definitive diagnosis of IDA; however, setting up these tests flanks with the more expensive and time-consuming options (2). Unfortunately, in a practical sense IDA and β-TT are classified as microcytic and hypochromic anemias, so mean corpuscular hemoglobin (MCH), the average mass of hemoglobin (Hg) per red blood cell (RBC), cannot differentiate all cases of IDA from β-TT (7).

Advances in information technology (IT) have led to computer-based decision-making system.

In this regard, artificial intelligence, and in particular smart systems are being applied and established across this spectrum (7). Therefore, recent developments in the field of artificial intelligence have led to the emergence of smart systems and the design of tools that can transfer and facilitate the expert knowledge into computer algorithms (12).

Artificial Neural Network (ANN) is an effective screening method for initial diagnosis and management of diseases. ANN is an advanced computer algorithm that is able to detect complex patterns in measured input variables that are not obvious to other forms of analysis. After processing the input data by several steps, the ANN shows the output by specifying a particular category in a classification. ANN has been successfully applied to a wide range of biomedical sciences. In a study produced a model based on data mining techniques of neural networks for predicting coronary artery disease and concluded that the model could identify both high risk patients and acceptable number of healthy subjects (13). Similarly, in a study examined the prediction premature birth in pregnant women via Assisted Reproductive

Technologies using ANN and concluded that designed neural network for predicting premature birth in pregnant women through Assisted Reproductive Technologies can be helpful in prevention of premature birth complications (14). Studies have shown that ANN has been able to accurately detect IDA and thalassemia with a significant degree of precision and sensitivity (8, 14).

Considering the high cost technique and clinical implications of differential diagnosis of iron-deficiency anemia from β-thalassemia trait, the aim of the present study was to develop a low cost, rapid, and reliable technique using...
neural network based model (Artificial Neural Network) for accurate and timely manner of differential diagnosis of iron-deficiency anemia from β-thalassemia trait.

1.1. Artificial Neural Networks (ANN)

Neural networks are dynamical systems developed to automate computer operations, including the extraction of new information similar to those of brain learning, in order to create and discover new knowledge. The platform of the ANNs are based on the processing of experimental data (input) and transfer the knowledge or logical data model (hidden layer) into a network structure (15). Figure 1, displays a simple diagram of the ANN in comparison with biological neural networks.

The network involves a large number of interconnected processing elements (neurons), which perform in parallel distributed processing systems (5, 16).

This computational process is very difficult or almost impossible with traditional methods. So, ANNs are required for extremely complicated explanation by processing information with tremendous computational power through computer program in a similar way the human brain does without human intervention. There are two different data phases in ANN, training and testing.

Training phase is a data set used to modify the weights of the nodes to form the model initially. It is based on calculations on the initial data, learn the general rules and data interfaces.

In the testing phase complete data set of the decision making is done without adequate data available in the initial stages of the design process.

Neural networks are one of the best techniques for identifying patterns or data trends and generating suitable model for prediction. Therefore, neural networks are widely used in medical diagnosis system (17). In order to reach an optimum solution and problem solving of variety of different disciplines, various network architecture and learning algorithm of ANN have been developed (5). Multilayer perception neural networks (MLPPNs) are nonparametric systems applied for the performance of wide range of detection and estimation tasks (5).

Multilayer perceptron (MLP) with back propagation training algorithm is the most widely powerful neural network used for prediction purposes and ease of interpretation. In the present study, an optimal MLP model with back propagation algorithm was developed for differentiate diagnosis of the β-thalassemia trait from iron deficiency anemia.

### Table 1. CBC data set explanation

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
<td>4-6 million/mm</td>
</tr>
<tr>
<td></td>
<td>is used to measure the number of oxygen-carrying blood cells in a volume of blood. Low/High RBC is diagnosed</td>
<td></td>
</tr>
<tr>
<td>HGB</td>
<td>Hemoglobin</td>
<td>12.6-16.1 g/dL</td>
</tr>
<tr>
<td></td>
<td>The protein in red blood cells that helps blood carry oxygen throughout the body</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>Mean Cell Volume</td>
<td>80-100 femtoliters (FL)</td>
</tr>
<tr>
<td></td>
<td>The average volume of a red blood corpusule (or red blood cell)</td>
<td></td>
</tr>
<tr>
<td>MCH</td>
<td>Mean Cell Hemooglobin</td>
<td>17-25 pico-gram(pg)</td>
</tr>
<tr>
<td></td>
<td>The average mass of hemoglobin (Hg) per red blood cell (RBC) in a sample of blood</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Architecture of the proposed MLP with BPNN

2. AIM

The aim of the present study was to develop a neural network based model (Artificial Neural Network) for accurate and timely manner of differential diagnosis of IDA and β-TT in comparison with traditional methods.

3. METHODS

3.1. Dataset

In the present study, the information of 268 patients referred to Padad private clinical laboratory at Ahvaz (Capital of Khuzestan province, Southwest of Iran) were selected and divided into two groups of IDA and thalassemia. The random sampling technique was used regard-
less of the sex and age criteria.

Studies have shown that the prevalence of β-thalassemia in Khuzestan province is between 2.7%–7.3% (18). The incidence of thalassemia is different among different tribes of Khuzestan, including Arabs, Lurs and Fars, but the results have shown that the ethnicity (Arab, Fars and Lurs) and the sexual characteristics of patients with thalassemia major have no significant effect on their survival (9).

Therefore, due to the high prevalence of β-thalassemia minor in the southern parts of Iran specially Khuzestan province, (19) Ahvaz was selected as the research site. Clinical and diagnostic tests showed that out of 268 subjects, 120 cases had thalassemia trait and 148 cases had IDA.

All patients with thalassemia had HbA2 levels above 3.5% and iron deficiency were confirmed by Ferritin test (< 8.0 ng/mL) in women and (< 28.0 ng/mL) in men.

3.2. Risk Factors

The data of Complete Blood Count (CBC) test considered for this study are presented in Table 1. These diagnostic indices are important for differentiation between patients of IDA and thalassemia, i.e. in each case the desired parameters show nonlinear changes.

3.3. Architecture Neural Network

Setting up a competent network architecture is one of the most challenging steps in the neural network model. In the proposed ANN model, the Back Propagation Neural Network (BPNN) was developed for the training algorithm. The topology of the BPNN consists of three layers: input, hidden, and output. In the present study, after different examination the optimal network architecture was comprised of four inputs, two outputs, and 70 neurons in the hidden layer.

The number of network layers, hidden layers, and termination criteria were determined based on the results of various experiments. In general, there is no general accepted theory for determining the optimal number of neurons in the hidden layers. Therefore, in order to find an optimal architecture, the number of different neurons in the hidden layers was considered and the prediction error was estimated for each network with minimal error and highest accuracy (Figure 2).

3.3.1. Forward Computing

In the process of forward computing, the input patterns put on to the neurons of the first layer are just a motive to the network and no calculation is available in the input layer. According to Figure 4, each neuron in the hidden layer settles a net input rate on the basis of all its input connections. These nodes are attached to each other, thereby the value of one node influences the other value. The relative influence of one node to another is identified by the “weight” consigned for each connection. The net input is computed by summing up the input values multiplied by their resultant weight. When the net input is analyzed, it is changed to an activated value.

The weight on the connection from the (i) neuron in the forward layer to the (j) neuron is revealed as w_ij. The output value of neuron (j) is calculated by the following formula:

\[ \text{net}_j = \sum_{i=0}^{n} W_{ij}x_i + x_0, \quad (1) \]

\[ Y_j = f_{ac}(\text{net}_j) \quad (2) \]

The (net_j) is the linear combination of x_i values multiplied by w_ij, x_0 remains constant as the bias. The parameter (n) is the extent of inputs to the (j) neuron, and (fact) stands for the activation of neuron (j).

In the present study, the hidden layer with Log-sig

![Figure 4. Transfer functions](Image)

(S-shaped curves) and Tan-sigmoid activation functions were applied for the activation of each hidden layer. The Log-sigmoid and Tan-sigmoid activation functions are given as follow:

\[ \text{Tan-Sigmoid:} \quad f(x) = \frac{2}{1+e^{-2x}} - 1 \quad (3) \]

\[ \text{Log-Sigmoid:} \quad f(x) = \frac{1}{1+e^{-x}} \quad (4) \]

The softmax function was used in the final layer for the activation of output layer. Given the importance of each parameter, the number of inputs, hidden layers and their neurons changed for each parameter, and then the results were evaluated. Measurement criteria such as sensitivity, specificity, and accuracy were calculated for each network.

\[ \sigma(x) = \frac{e^x}{\sum_{k=1}^{K} e^x} \quad \text{for } j=1,...,k \quad (5) \]

3.4. Implementing of Neural network

The development and the training of the network model were conducted by MATLAB software, Ver. 2018. In the process of MATLAB function the IDA and thalassemia target groups were divided into three sets: training (70%), validation (15%), and testing (15%). The mean accuracy of diagnosis and the results were presented and summarized in the confusion matrix. This process was iterated ten times for each set of features studied.

4. RESULTS

In the present study, the accuracy, sensitivity, and specificity in each iteration in the test group were calculated.
All of the performance metrics were computed based on the confusion matrix. The confusion matrix is a 2 × 2 matrix for envisioning the behavior of models in the managed classification contexts. In the present study, the resulting confusion matrix reports the number of true positives (#TP), true negatives (#TN), false positives (#FP), and false negatives (#FN) as follow:

\[
\begin{array}{c|c|c}
\text{Actual class} & \text{Thalassemia} & \text{IDA} \\
\hline
\text{Predictive class} & \text{#TP} & \text{#FP} \\
\text{Thalassemia} & & \\
\text{IDA} & \text{#FN} & \text{#TN} \\
\end{array}
\]

TP: Thalassemia is diagnosed and properly classified in thalassemia group.

TN: IDA is diagnosed and properly classified in IDA group.

FP: IDA is diagnosed and incorrectly classified in thalassemia group.

FN: Thalassemia is diagnosed and incorrectly classified in IDA group.

TP and TN are values that must be maximized in binary subjects, because these two values correctly identify the algorithm. Table 2 represents the mean accuracy, sensitivity, and specificity of the proposed model dataset (10 iteration). The mean accuracy of performance evaluation of ANN in three datasets of training, validation, and testing was 90.1%, 92.5%, and 91.5%, respectively.

Accuracy is the most important criterion for determining the efficiency of an algorithm, indicating the ratio of number of correct predictions to the total number of input data.

Sensitivity stands for the ability of the algorithm to accept the correct results of thalassemia.

Specificity = True negative/ (True negative + False positive), indicating the effectiveness of the model in predicting the true absence of thalassemia.

<table>
<thead>
<tr>
<th>Mean Dataset</th>
<th>Accuracy (%)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training</td>
<td>90.01%</td>
<td>90.4%</td>
<td>89.8%</td>
</tr>
<tr>
<td>Validation</td>
<td>92.5%</td>
<td>93.13%</td>
<td>92.33%</td>
</tr>
<tr>
<td>Testing</td>
<td>91.5%</td>
<td>89.9%</td>
<td>92.81%</td>
</tr>
</tbody>
</table>

Table 2. The mean value of performance evaluation of ANN in three datasets of training, validation, and testing

Figure 5 shows the performance of the proposed ANN model in the training process. According to Figure 5, the validation performance was reached to Mean Squared Error (MSE) of 0.13309 after 27 epochs. The training set error converged toward validation error.

5. DISCUSSION

IDA and β-thalassemia trait are among the most common types of microcytic hypochromic anemias. The differential diagnosis of IDA from β-thalassemia trait are important for therapeutic treatment plan. β-thalassemia trait is diagnosed with the microcytosis and increased level of Hemoglobin A2 (20). Low serum levels of ferritin and content of iron are the most criteria for diagnosis of IDA (21).

Different indices and formulas of CBC parameters and screening program have been designed for diagnosis of signs and symptoms of β-thalassemia minor from IDA in the early stages of the project (Table 3). This type of computation models comes up with the error and complexity and do not provide the appropriate sensitivity and reliability, so that represent different results in different conditions.

The risk of diagnosis in these methods can be attributed to differences in inclusion and exclusion criteria in clinical practice, similarity between the age group and sex, the difference in HbA2 and serum hemoglobin and ferritin levels. The controversy in the divulges of similarities/differences make it hard to compare results across studies and could report the contradictory results (11).

<table>
<thead>
<tr>
<th>Method</th>
<th>Accuracy (%)</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>MCH/RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mentzer index</td>
<td>74.2</td>
<td>70.5</td>
<td>75.7</td>
<td>MCV/RBC</td>
</tr>
<tr>
<td>Srivastava formula</td>
<td>73.8</td>
<td>75.0</td>
<td>73.4</td>
<td>MCH/RBC</td>
</tr>
<tr>
<td>Ehsani formula</td>
<td>76.7</td>
<td>70.0</td>
<td>79.5</td>
<td>MCV-10 *RBC</td>
</tr>
<tr>
<td>New formula</td>
<td>86.9</td>
<td>87.9</td>
<td>84.7</td>
<td>[80-MCV]*[27-MCH]</td>
</tr>
<tr>
<td>Mentzer index</td>
<td>— — —</td>
<td>80.3</td>
<td>90.9</td>
<td>MCV/RBC</td>
</tr>
</tbody>
</table>

Table 3. Performance of various formulas in differentiating BTMi from other causes of microcytic hypochromic anemia

As well as, since these formulas cannot be used for children, pregnant women, and patients with iron deficiency anemia, CBC and RBC indices are not trustworthy tools for distinguishing β-TT from IDA (22).

Table 3 displays the accuracy, sensitivity, and specificity of performance of various formulas in differentiating BTMi (β-TT/IDA) from other causes of microcytic hypochromic anemia. These formulas and indices provide differential diagnostic error over 13%. However,
Table 4. Comparison of the proposed model with the current studies

<table>
<thead>
<tr>
<th>Accuracy (%)</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Method</th>
<th>Year</th>
<th>Diagnosis</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>90.7</td>
<td>95.6</td>
<td>87.1</td>
<td>ANFIS</td>
<td>2012</td>
<td>Iron Deficiency Anemia and Iron Serum</td>
<td>Azarkhish et al. (24)</td>
</tr>
<tr>
<td>96.3</td>
<td>95.6</td>
<td>96.8</td>
<td>ANN</td>
<td>2012</td>
<td>Iron Deficiency Anemia and Iron Serum</td>
<td>Azarkhish et al. (24)</td>
</tr>
<tr>
<td>92.5</td>
<td>92.0</td>
<td>95.0</td>
<td>MLP</td>
<td>2002</td>
<td>Thalassemic pathologies</td>
<td>Amendolia et al. (25)</td>
</tr>
<tr>
<td>89.0</td>
<td>83.0</td>
<td>95.0</td>
<td>SVM</td>
<td>2003</td>
<td>Thalassemia screening</td>
<td>Amendolia et al. (26)</td>
</tr>
<tr>
<td>95.0</td>
<td>93.0</td>
<td>97.0</td>
<td>KNN</td>
<td>2003</td>
<td>Thalassemia screening</td>
<td>Amendolia et al. (26)</td>
</tr>
<tr>
<td></td>
<td>91.0</td>
<td>93.0</td>
<td>RBF</td>
<td>2013</td>
<td>Screening of thalassemia</td>
<td>Masala et al. (27)</td>
</tr>
<tr>
<td></td>
<td>73.0</td>
<td>89.0</td>
<td>PNN</td>
<td>2013</td>
<td>Screening of thalassemia</td>
<td>Masala et al. (27)</td>
</tr>
<tr>
<td></td>
<td>91.0</td>
<td>80.0</td>
<td>KNN</td>
<td>2013</td>
<td>Screening of thalassemia</td>
<td>Masala et al. (27)</td>
</tr>
<tr>
<td>85.95</td>
<td>87.9</td>
<td>84.0</td>
<td>Math</td>
<td>2015</td>
<td>Discriminating between β-Thalassemia Minor and other Microcytic Hypochromic</td>
<td>Bordbar et al. (23)</td>
</tr>
<tr>
<td>92.5</td>
<td>92.33</td>
<td>93.13</td>
<td>ANN</td>
<td>2018</td>
<td>Discrimination between Iron Deficiency Anemia (IDA) and β-Thalassemia Trait (β-TT)</td>
<td>Proposed model</td>
</tr>
</tbody>
</table>

Table 4. Comparison of the proposed model with the current studies

more laboratory testing is needed to make definitive diagnosis. These formulas also produce false positives results in patients with medical condition of pregnancy, malnutrition, rheumatoid arthritis, tuberculosis, kidney failure, and malaria (23).

Table 4 shows the results of the various models for discrimination between IDA and β-TT using ANN. In a study achieved to the accuracy of 96.3% using Neuro-Fuzzy Inference Systems (ANFIS) which was more than the value of the proposed model of the present study. However, the high accuracy of the ANFIS model for detecting IDA was obtained without the analysis of β-thalassemia data (24). The results of the other similar studies also suggested a higher performance error compared to the accuracy obtained in the proposed model of the present study (23, 25-27). Since, verification and validation of ANN-based models are one of the main challenges for problem solving at a meaningful prediction unique and obtaining comprehensive outcome, the mean performance of the proposed model in different performances and displayed acceptable results in the dataset of testing and validation. Similarly, the sensitivity and specificity with close error in fitting the data indicate the proper functioning of the model in the diagnosis of diseases. The results of the similar studies displayed poorer performance. These results may be biased because of the absence of one of the variables of IDA and β-thalassemia in data analysis.

While in the proposed model, due to the similarity of the factors involved in the IDA and β-thalassemia, differential diagnosis of the two types of diseases was examined. However, more differential diagnostic model in the more complex conditions should be examined.

6. CONCLUSION

The present study was set out to examine the application of ANN for differential diagnosis of β-thalassemia and IDA. The performance of these model in the categorization of various forms of anemia was scrutinized. The accuracy results of the proposed ANN model introduced in the present study have shown better outcome based on CBC criteria compared to original methods. In this intelligent model, optimal settings of ANN parameters with little complexity and highly optimized learning rate and moment term has been realized. The proper function of the model makes it possible to use it as a safe alternative method for accurate, timely manner, and cost-less diagnosis method than existing methods. Therefore, the reports provided by this model along with clinical signs and symptoms, and laboratory tests provide physicians powerful and accurate tools to diagnose diseases.

It is recommended that to use this model of ANN as a module on the cell counting instruments to directly diagnose the parameters of CBC in differential diagnosis of iron deficiency anemia from β-thalassemia trait.

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