Trends in Clinical and Medical Sciences

PSR Press

Article

Derangement of gonadal hormone and its relation with oxidative stress for β-thalassemia patients

Debleena Basu[1,*], Rupal Sinha[2], Saswata Sahub[2], Jyotsna Malla[3] and Partha Sarathi Ghosal[2]

[1] Department of Biochemistry, R.G. Kar Medical College & Hospital Kolkata, Kolkata, 700004, West Bengal, India.
[2] School of Water Resources, Indian Institute of Technology Kharagpur, Kharagpur, 721302, India.
[3] Computer Science and Engineering, Vellore Institute of Technology, Vellore, 632014, India.
* Correspondence: psghosal@swr.iitkgp.ac.in

Academic Editor: Augustine Ibegbu
Received: 21 May 2023; Accepted: 21 October 2023; Published: 13 November 2023.

Abstract: Background: Background: Enhanced iron levels in patients afflicted with β-thalassemia induces oxidative stress, which restrains the secretion of gonadal and pituitary hormones. The associated severity level based on several hormones and oxidative stress biomarkers is not been demonstrated so far. **Method:** The present study encompasses the employment of hierarchical clustering and different classifiers for determining the severity of the disease based on the analyzed clinical parameters in the study population. Furthermore, the hormonal parameters along with ferritin levels were used as input parameters for the prediction of the oxidative stress biomarkers ([Malondialdehyde (MDA) and protein carbonyl] through neural networks. **Result:** A Significant negative correlation was observed between the oxidative stress biomarkers and the hormonal levels in both the female and male datasets of the case group. The clustering results depicted that the datasets corresponding to high oxidative stress biomarkers with very low gonadal hormones represented severe levels of the disease. Support vector machine outperformed the other classifiers in the case of the male dataset. The neural network efficiently predicted female and male models' MDA and protein carbonyl values. High Fisher's F-value (2042.035 to 6353.659) and low p-value (<0.001) established the significance of each model. **Conclusion:** The proposed framework can be used as a real-life decision tool for medical professionals to diagnose and treat β-thalassemia from a proper classification of the severity of the disease. Furthermore, the passive determination of some critical blood parameters may avoid the complex analytical procedure and its high cost.

Keywords: Pituitary hormones; Machine learning; Ferritin; MDA; Protein Carbonyl

1. Introduction

Abnormality in the genes responsible for regulating hemoglobin formation results in an autosomal recessive trait known as Thalassemia [1]. β-thalassemias are one of the most common inherited hemoglobinopathy in the globe [2]. The mutations induce absent or declined synthesis of β-globin protein in the erythropoietic cells [3]. They are prevalent among the Mediterranean population, the Middle East, and Central and Southeast Asia. β-thalassemias display different genetic mutations, giving rise to a diverse range of symptoms in patients from asymptomatic expression (β-thalassemia minor) and mild clinical anemia (β-thalassemia intermedia) to severe Cooley's anemia [4]. Cooley's anemia refers to β-thalassemia major, the fatal form of thalassemia requiring medical attention in the early years of life [5]. Infants afflicted with thalassemia significantly experience skeletal abnormalities, stunted growth, and severe ineffective erythropoiesis and, therefore, rely on RBC transfusions for survival [6]. The regular RBC transfusions expose the β-thalassemia major patients to hemosiderosis [3]. Iron accumulation at elevated levels in the body leads to generating reactive oxygen species (ROS), propagators of oxygen-related damage [7]. MDA and protein carbonyl are well-recognized oxidative stress biomarkers of lipid peroxidation and protein oxidation, respectively, which have been observed to be significantly enhanced in the β-thalassemic patients [8,9]. Increased oxidative stress in *beta*-thalassemia-affected individuals has been related to endocrine complications in such patients due to the disruption in the secretion of gonadal and pituitary hormones [10]. Consequently,

such patients experience delayed puberty, hypogonadism, and other endocrine problems, which create a barrier to the desire for parenthood among these patients [10,11]. β-thalassemia major female patients generally suffer from hypogonadotropic hypogonadism associated with ovulation, amenorrhea, and infertility [12]. Some studies have reported oligoasthenospermia and sperm DNA damage and reduced testicular function in transfusion-dependent β-thalassemia male patients [13–15]. Early assessment, detection, and treatment of endocrine complications can prevent late irreversible sequelae and increase the chances of successful reproduction [16]. Therefore, gonadal hormonal parameters (estradiol, progesterone in females, and testosterone in males) together with serum ferritin (parameter for assessment of iron overload), oxidative stress biomarkers should be routinely assessed in patients afflicted with β-thalassemia. Several studies have depicted a significant negative correlation between gonadal hormones and iron-induced oxidative stress biomarkers in β-thalassemia major patients [17,18]. Therefore, the acuteness of this disease may be classified considering the gonadal hormonal parameters, oxidative stress biomarkers, and ferritin levels of these patients. Furthermore, many countries quantify oxidative stress biomarkers through costly procedures. In this context, a wide application of machine learning techniques have been observed in various field of medical science, such as image processing [19] and speech therapy [20]. Furthermore, numerous classification and clustering algorithms have been used by many researchers to analyze clinical challenges [21–26]. The artificial neural network has long been adopted as a classification and prediction tool, from detecting brain injury to screening chromatograms as essential applications in the medical field [27,28]. In our previous study, we developed a framework based on machine learning techniques for assessing the severity of thalassemic disease based on oxidative stress biomarkers, ferritin level, and trophic hormone parameters [5]. However, the impact of gonadal hormones and the oxidative stress biomarkers in thalassemic patients needs to be addressed. In the present study, an extensive primary dataset was prepared through the estimation of the degree of hypogonadism by the assessment of pituitary and gonadal hormones [Luteinizing hormone (LH), Follicle Stimulating Hormone (FSH), estradiol, progesterone in female and LH, FSH, testosterone in male]. Furthermore, the level of MDA and protein carbonyl has been assessed to study the impact of oxidative stress markers on the gonadal hormonal parameters. Moreover, the present study employs the use of machine learning techniques that serve a twofold purpose: (i) Categorizing the study population based on the degree of severity of β-thalassemia disease using gonadal, pituitary hormones through hierarchical clustering and classification algorithms; (ii) Passive measurement of MDA and protein carbonyl using ferritin, gonadal, pituitary hormones, as surrogate parameters through neural networks.

2. Materials and Methods

2.1. Study design

The study involved the selection of two groups of the population comprising case (β-thalassemic patients) and control (healthy individuals). Subsequently, 55 (28 female and 27 male) and 50 (27 female and 23 male) individuals were chosen for case and control, respectively, and blood serum samples for both groups were collected for every individual. The framework of the study protocol is represented in Figure 1. The blood serum samples were determined for gonadal hormones (estradiol, progesterone in females, and testosterone in males), pituitary hormones (FSH, LH), oxidative stress biomarkers, and ferritin. The correlation between oxidative stress biomarkers and gonadal hormone parameters was established with statistical analysis. Furthermore, a hierarchical clustering technique was employed to categorize the female and male datasets in different severity levels. Subsequently, different classification algorithms, such as Naive Bayes, K-Nearest neighbor (KNN), and Support Vector Machine (SVM), were used for the classification of the severity level of the thalassemia as the confirmatory analysis of the arrived clusters. A neural network model was also employed to predict oxidative stress biomarkers using the input of relevant parameters. The model adequacy was assessed by ANOVA technique and error analysis.

2.2. Sample population and study parameters

This study's sample population consisted of cases comprising β-thalassemia diagnosed patients (based on clinical or HPLC or other laboratory results) attending the Department of Pathology in Calcutta National Medical College, Kolkata. On the other hand, control is comprised of healthy individuals. The inclusion

and exclusion criteria and the collection procedure of serum samples have been referred to in Section S1 of the supplementary material (SM). The Cases and Control sample size was 55 (28 female and 27 male) and 50 (27 female and 23 male), respectively. The oxidative stress biomarkers, such as serum MDA and protein carbonyl, were detected in the laboratory. The detailed procedure has been referred to in section S2 of the SM. Gonadal hormone levels, such as serum estradiol and progesterone in females and testosterone in males, and pituitary hormone levels, such as serum FSH and LH levels together with ferritin levels, were all estimated by the ELISA method. After determining the study parameters, the SPSS 17.0 window was used to analyze the data statistically. The significance of data was analyzed using the man-Whitney test, whereas Spearman's correlation study analyzed the correlation between the study parameters.

2.3. Employment of machine learning techniques

The complete database of 105 individuals has been segregated into two datasets based on their gender-female and male datasets comprising 55 and 50 samples, respectively. The detected gonadal hormone parameters- estradiol, progesterone for females, and testosterone for males have been used as input parameters, along with serum ferritin, oxidative stress markers, and pituitary hormonal levels. Before running both the models against the dataset, data with all incomplete instances, irrelevant attributes, and duplicate data records were removed manually for the entire dataset. Furthermore, all attributes were scaled to the same interval for the attributes to have the same range of values and to avoid the impact of attributes with a wider range of values on the training process. In this work, all numeric features are normalized using 'min-max' normalization and, therefore, scaled to the range [0, 1].

2.3.1. Assessment of severity via hierarchical Clustering

Hierarchical clustering has been employed to find the clusters within the dataset to differentiate the individuals according to the severity of the disease. In this clustering technique, each data point is reckoned as an individual cluster initially [29]. Subsequently, similar clusters merge, and the process keeps repeating until a single cluster or K clusters are created. Different approaches are used to determine cluster similarity, such as the max, min, group average, Ward's method, and distance between centroids. The distance between the two merging clusters is recorded and represented graphically through dendrograms. They have been used to find the optimal number of clusters. In this study, agglomerative hierarchical clustering has been used. "AgglomerativeClustering" is imported from sklearn library [30] for both datasets. The "Train-test-split" function is imported from sklearn library [30] to divide the data into training and testing for both datasets. For both the models, 82% of the dataset was used for training and testing while 18% was kept for validation. Clusters have been appended concerning the study parameters, and the datasets were categorized based on the disease severity level in both models. The influence of each parameter on the cluster was analyzed.

2.3.2. Classification algorithms

After clustering, different classifiers, such as k-nearest neighbors (KNN), Naive Bayes, and Support vector machine (SVM), were employed for the classification of the severity level of the thalassemia disease as the confirmatory analysis of the arrived clusters. KNN is an instance-based classification algorithm where the unknown instances are classified based on the nearest known instances in the feature space. It predicts the class of the test sample according to the K training samples that are the nearest neighbors to the test sample. It assigns it to that category, which has the largest category probability [31]. KNN classifiers perform well in multiclass simultaneous problem solving [1]. The K value in KNN denotes the count of the nearest neighbors and, therefore, impacts the classifier's performance. Naive Bayes classifiers are probabilistic learning methods, which are designed based on Bayesian theorem [32]. The classifier works on a strong assumption that the impact of a certain feature on a given class is independent of the existence of any other feature. The model is easily built without complicated iterative parameter estimation [33]. The algorithm works well in multiclass prediction. Support Vector Machine (SVM) refers to a linear method in a very high dimensional feature space that is nonlinearly related to the input space, widely used for classification and regression problems [34]. In the training data, SVM differentiates between the members of the two classes for estimating the best classification function. The SVM algorithm aims to find a hyperplane in an N-dimensional space that separately classifies the data points [35]. Many possible hyperplanes can be chosen to segregate the two

classes of data points. The objective is to find a plane having the maximum distance between data points of both classes. The female and male datasets were trained using the three classifier models, and the code was run on Python. "KNeighborsClassifier," "GaussianNB," "SVC" were imported from sklearn library [30] for multiclass classification, and therefore, the dataset has been classified.

2.3.3. Determination of oxidative stress biomarkers through shallow neural network

Machine learning approach, such as artificial neural networks, is one of the most preferred choices for prediction modeling. In this technique, the model is trained by learning from the data instead of from the rule. The universal mapping capacity, robustness, fault tolerance, and parallel processing of the dataset make ANN a versatile and promising tool for multivariate modeling [36,37]. The major influencing parameters for β-thalassemia were considered serum ferritin, FSH, LH, estradiol, and progesterone for females and serum ferritin, FSH, LH, and testosterone for males to model the oxidative stress biomarkers (MDA and protein carbonyl) as response. In this context, we have developed separate prediction models for MDA and protein carbonyl for each category of male and female groups. The network architecture for the prediction models considering the input and the response parameters is shown in Figures 2(a) and 2(b). The case and control datasets were merged by assigning a categorical variable as 1 and 2, respectively. Here, the models are framed from a composite database of case and control. In contrast, some data are separated to validate the model with different datasets as new data unrelated to the model preparation. The model was optimized by varying the important parameters pertaining to its configuration, such as the number of nodes in the hidden layer, transfer function, etc. The number of nodes in the single hidden layer varied from 1 to 20 in each model with 500 iterations to optimize the response. Three non-linear transfer functions, viz. purelin, tangent sigmoid, and log sigmoid, were considered to obtain an optimum response. The Levenberg-Marquardt (trainlm) optimization was used as a training algorithm. The normalization of input and targets was performed by 'mapminmax' function. The complete dataset was split into training, testing, and cross-validation with 70%, 15%, and 15%, respectively. The coefficient of determination (R2) and root mean square error function (RMSE) were chosen as performance estimators for the prediction model. The coding of neural network analysis was performed in MATLAB, R2019a.

3. Result

3.1. Statistical analysis

The results of all the study parameters for cases and control for both female and male datasets are presented in Table S1-S4 of the SM. In contrast, the statistical analysis results are presented in Table 1-3. Table 1 compares study parameters in females and males using the Mann-Whitey test. The results depict enhanced serum ferritin levels in the case group of both females and males. On the other hand, the FSH, LH, estradiol, and progesterone were significantly low in cases compared to the control with p-value <0.001. Similarly, in males, pituitary hormone levels and gonadal hormone, viz. testosterone levels, were significantly lower in the β-thalassemic patients (Table 1). Tables 2 and 3 represent the results of nonparametric Spearman's analysis of the case groups of the female and male datasets, respectively. It can be observed that lipid peroxidation and protein oxidation products, i.e., MDA and protein carbonyl, significantly correlated with body iron deposition, i.e., serum ferritin and hormonal parameters. Furthermore, a negative correlation was observed between ferritin and gonadal hormone, i.e., estradiol & progesterone in females. Similarly, in the male dataset, ferritin levels depicted a significant negative correlation with testosterone levels and other hormonal parameters (Table 3). Similar findings have been reported in other studies [17,38].

Table 1. Comparison of the parameters between cases and controls by Mann-Whitney test

For female					
Parameters	Serum Ferritin (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)	Estradiol (pg/ml)	Progesterone (ng/ml)
Case Mean	41.50	16.84	15.46	16.50	16.04
Rank					
Median	2695.50	1.25	0.80	4.00	1.01
Control Mean	14.00	39.57	41.00	39.93	40.41
Rank					
Median	75.00	8.20	8.40	28.41	5.83
Z	-6.36	-5.26	-5.91	-5.42	-5.64
p value*	<0.001	<0.001	<0.001	<0.001	<0.001
For male					
Parameters	Serum Ferritin (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)	Testosterone (ng/ml)	
Case Mean	37.00	14.33	14.00	14.52	
Rank					
Median	3672.00	1.21	0.97	1.52	
Control Mean	12.00	38.61	39.00	38.39	
Rank					
Median	91.00	7.30	7.90	6.89	
Z	-6.04	-5.87	-6.04	-5.77	
p value*	<0.001	<0.001	<0.001	<0.001	

*p value is considered to be significant at a level of $p < 0.05$ for a 95% confidence interval

Table 2. Nonparametric Spearman's correlation analysis between test parameters in cases of female

Analyst		MDA	Protein Carbonyl	Serum Ferritin	FSH	LH	Estradiol	Progesterone
MDA	r	1.000	0.713**	0.755**	-0.697**	-0.686**	-0.699**	-0.566**
	p	.	<0.001	<0.001	<0.001	<0.001	<0.001	0.002
Protein	r	0.713**	1.000	0.655**	-0.449*	-0.520**	-0.312	-0.324
Carbonyl	p	<0.001	.	<0.001	0.017	0.005	0.106	0.093
Serum	r	0.755**	0.655**	1.000	-0.383**	-0.439*	-0.570**	-0.516**
Ferritin	p	<0.001	<0.001	.	0.044	0.020	0.002	0.005
FSH	r	-0.697**	-0.449*	-0.383**	1.000	0.608**	0.548**	0.308
	p	<0.001	0.017	0.044	.	0.001	0.003	0.111
LH	r	-0.686**	-0.520**	-0.439*	0.608**	1.000	0.456*	0.664**
	p	<0.001	0.005	0.020	0.001	.	0.015	<0.001
Estradiol	r	-0.699**	-0.312	-0.570**	0.548**	0.456*	1.000	0.218
	p	<0.001	0.106	0.002	0.003	0.015	.	0.266
Progesterone	r	-0.566**	-0.324	-0.516**	0.308	0.664**	0.218	1.000
	p	0.002	0.093	0.005	0.111	<0.001	0.266	

r- Correlation coefficient p value is considered to be significant at a level of $p < 0.05$ for 95% confidence interval.

** Bold p values indicate statistically significant difference between two parameters in female case group of β-thalassemia major.

Table 3. Nonparametric Spearman's correlation analysis between test parameters in cases of male

Analyst		MDA	Protein Carbonyl	Serum Ferritin	FSH	LH	Testoserone
MDA	r*	1.000	0.648**	0.735**	-0.688**	-0.696**	-0.632**
	p	.	<0.001	<0.001	<0.001	<0.001	<0.001
Protein	r	0.648**	1.000	0.673**	-0.375	-0.193	-0.303
Carbonyl	p	<0.001	.	<0.001	0.054	0.335	0.124
Serum	r	0.735**	0.673**	1.000	-0.446*	-0.314**	-0.554**
Ferritin	p	<0.001	<0.001	.	0.020	0.111	0.003
FSH	r	-0.688**	-0.375	-0.446*	1.000	0.493**	0.386*
	p	<0.001	0.054	0.020	.	0.009	0.047
LH	r	-0.696**	-0.193	-0.314**	0.493**	1.000	0.548**
	p	<0.001	0.335	0.111	0.009	.	0.003
Testosterone	r	-0.632**	-0.303	-0.554**	0.386*	0.548**	1.000
	p	<0.001	0.124	0.003	0.047	0.003	.

*r -Correlation coefficient p value is considered to be significant at a level of $p < 0.05$ for 95% confidence interval.

** Bold p values indicate statistically significant difference between two parameters in male case group of β-thalassemia major.

Table 4. Performance of classification algorithms

Female dataset						
Algorithm	Accuracy from test set	Accuracy from training set	Accuracy after K cross validation	Standard deviation	Specificity	Sensitivity
KNN(N_Neighbors= 5)	0.66	0.85	77.78%	3.93%	1.00	1.00
KNN(N_Neighbors= 1)	0.66	0.76	91.67%	6.80%	1.00	1.00
Naive Bayes	1.0	1.0	83.33%	11.79%	1.00	1.00
SVM	0.88	0.84	88.89%	7.86%	0.5	1.00
Male dataset						
Algorithm	Accuracy from test set	Accuracy from training set	Accuracy after K cross validation	Standard deviation	Specificity	Sensitivity
KNN(N_Neighbors= 5)	0.875	0.968	90.91%	7.42%	1.0	1.0
Naive Bayes	1.00	1.00	93.94%	4.29%	1.0	1.0
SVM	1.00	1.00	100.00%	0.00%	1.0	1.0

Table 5. ANOVA of training and validation dataset of neural network model

Model	Degree of Freedom	Sum of squares (SS)	Mean square (MS)	F value	p value
MDA/female					
Overall					
Regression	1	739.063	739.063	6353.659	<0.001
Residual	42	4.885	0.116		
Total	43	743.949			
Validation					
Regression	1	114.264	114.264	6.912	<0.001
Residual	7	115.727	16.532		
Total	8	229.992			
Protein Carbonyl/female					
Overall					
Regression	1	11.822	11.822	2042.035	<0.001
Residual	42	0.243	0.006		
Total	43	12.065			
Validation					
Regression	1	0.720	0.720	2.199	<0.001
Residual	7	2.291	0.327		
Total	8	3.011			
MDA/male					
Overall					
Regression	1	1069.430	1069.430	2297.632	<0.001
Residual	38	17.687	0.465		
Total	39	1087.117			
Validation					
Regression	1	145.173	145.173	10.316	<0.001
Residual	6	84.432	14.072		
Total	7	229.605			
Protein carbonyl/male					
Overall					
Regression	1	14.821	14.821	3108.510	<0.001
Residual	38	0.181	0.005		
Total	39	15.002			
Validation					
Regression	1	4.526	4.526	119.218	<0.001
Residual	6	0.228	0.038		
Total	7	4.754			

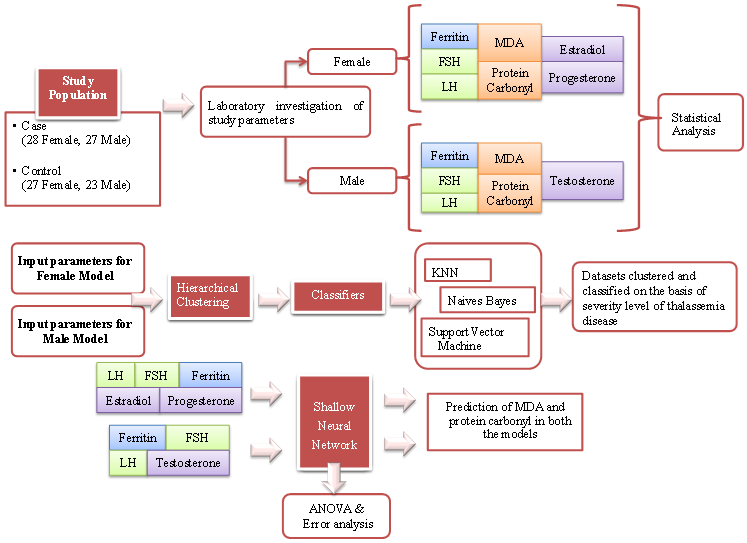

Figure 1. Framework of the study

3.2. Clustering results

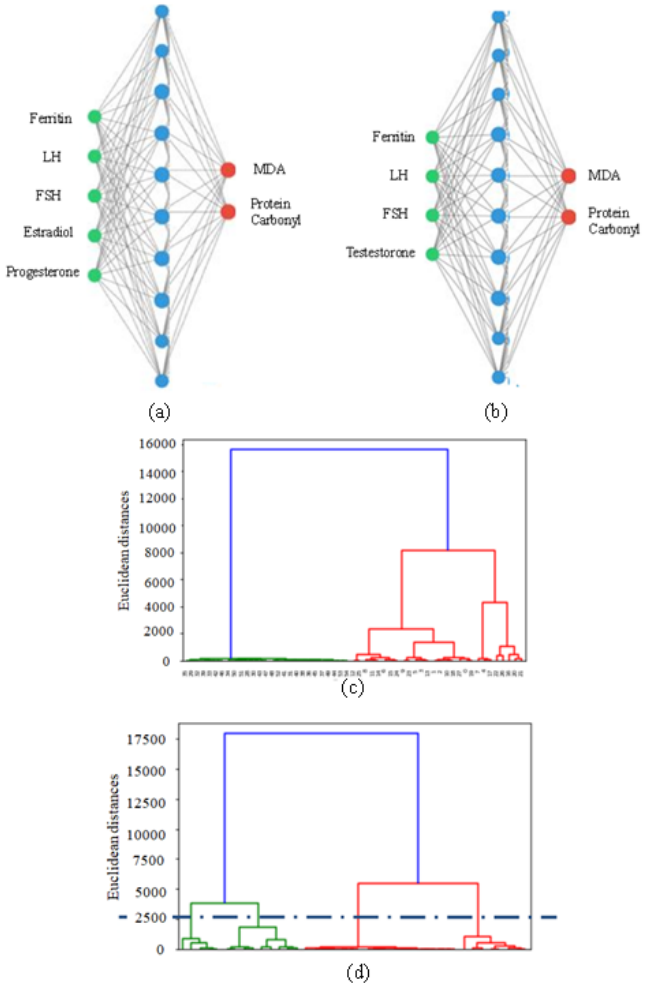

Figure 2. . (a) Neural Network architecture for Female dataset, (b) Neural Network architecture for Male dataset (c) Dendrogram of Hierarchical clustering for Female dataset, (b) Dendrogram of Hierarchical clustering for Male dataset

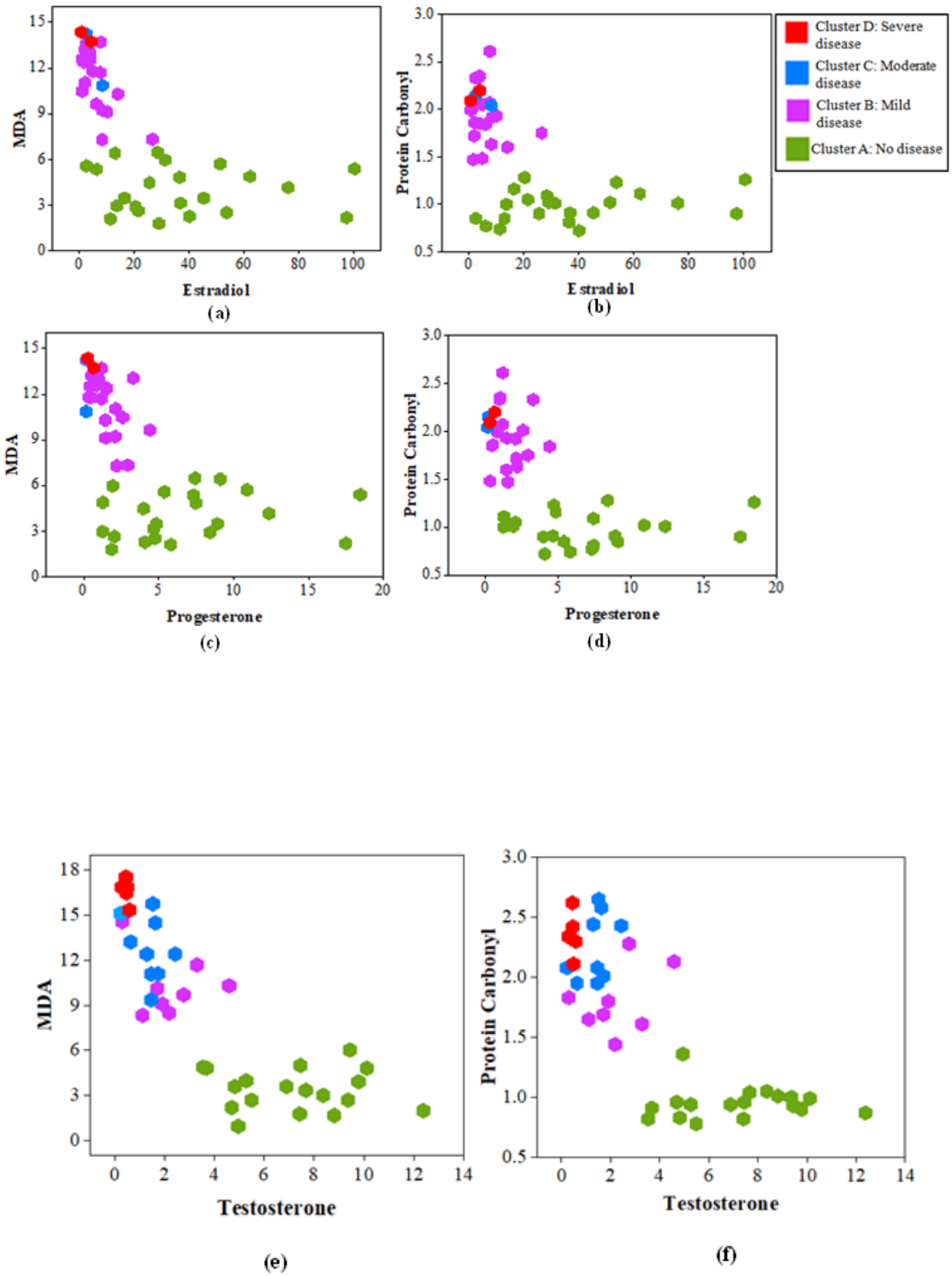

Figure 3. Hierarchical clustering generated results of female and male dataset (a) MDA vs. Estradiol, (b) Protein carbonyl vs. Estradiol, (c) MDA vs. Progesterone, (d) Protein carbonyl vs. Progesterone, (e) MDA vs. Testosterone, and (f) Protein carbonyl vs. Testosterone

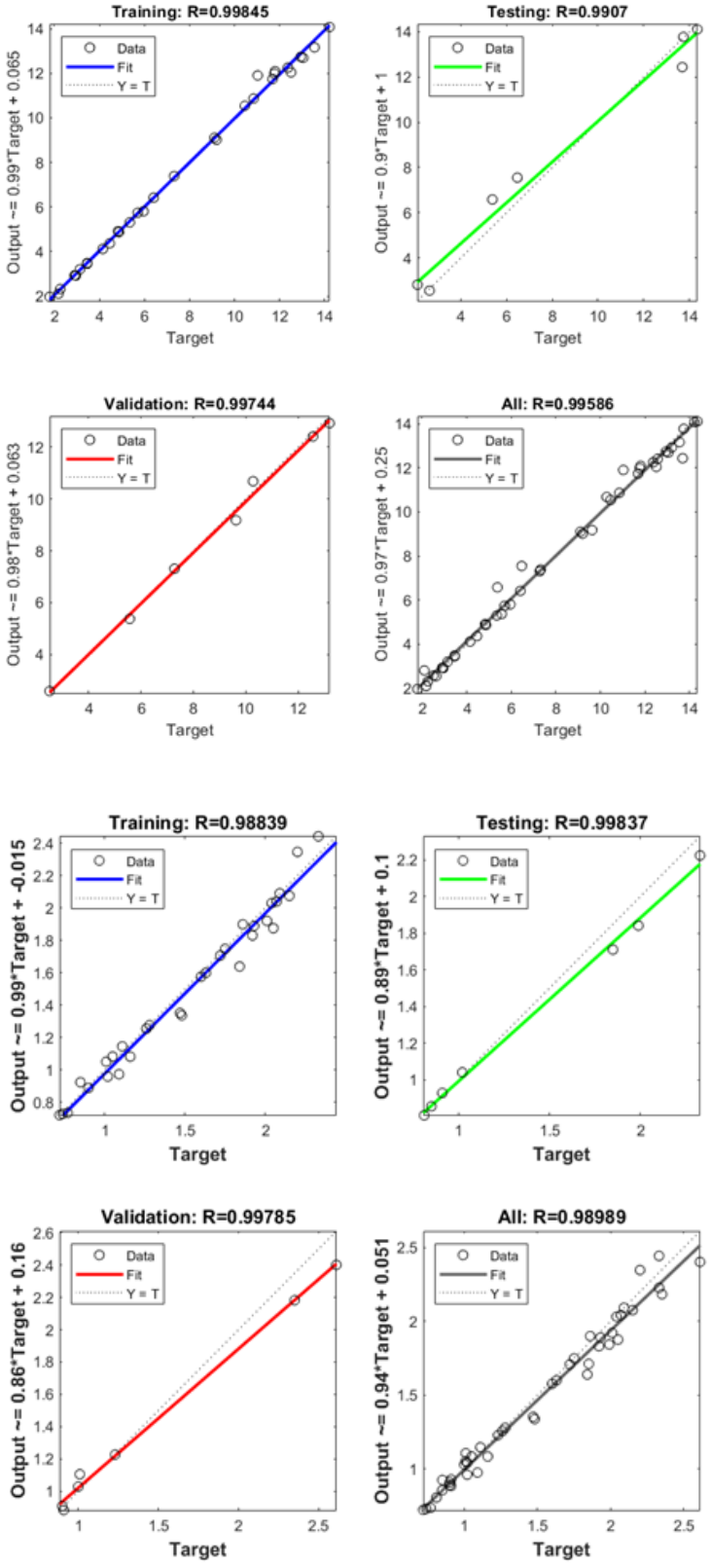

Figure 4. ANN modeling results of female dataset (a) Values of R2 for training, testing, validation, and overall dataset for MDA model, (b) Values of R2 for training, testing, validation, and overall dataset for Protein carbonyl model

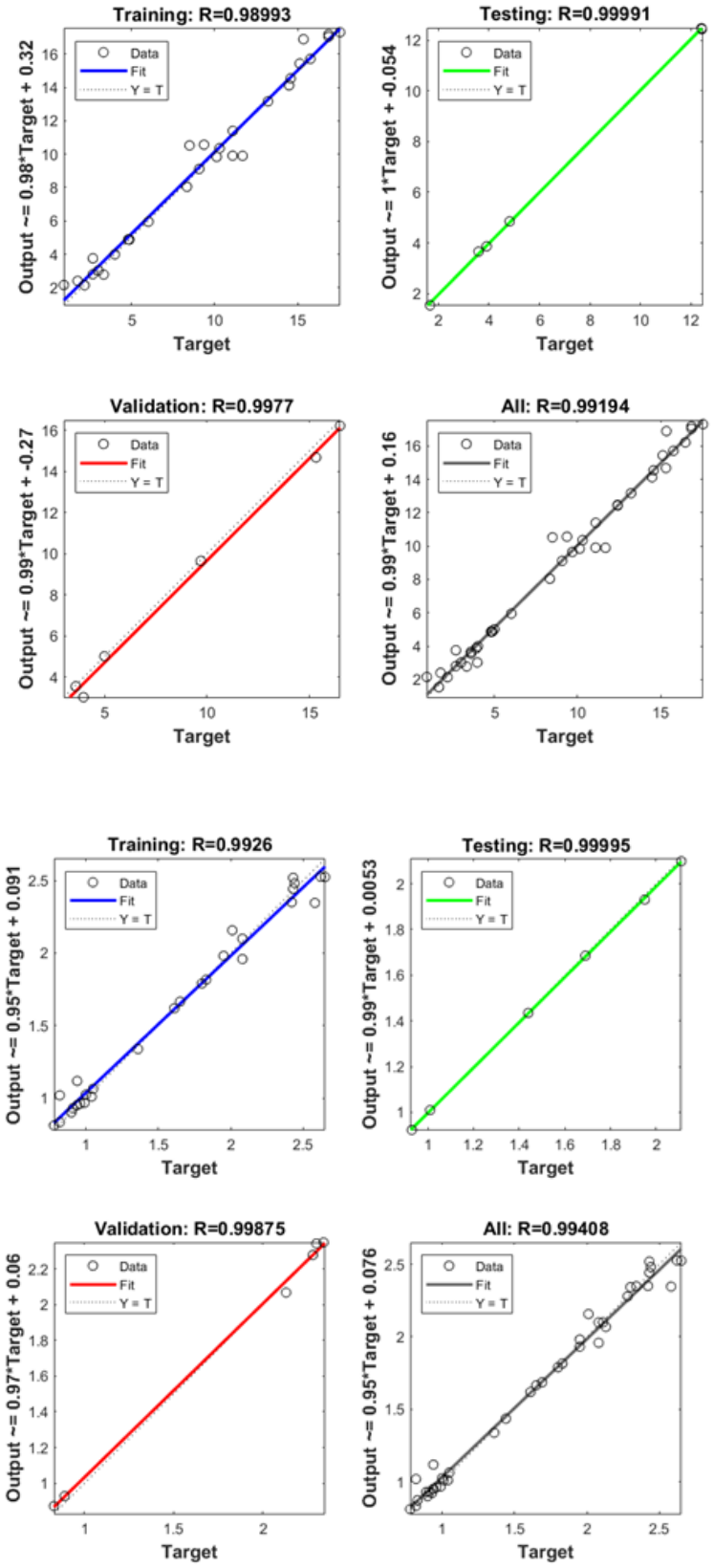

Figure 5. ANN modeling results of male dataset (a) Values of R2 for training, testing, validation, and overall dataset for MDA model, (b) Values of R2 for training, testing, validation, and overall dataset for Protein carbonyl model.

The dendrograms for both female and male models of hierarchical clustering are represented by Figures 2(c) and 2(d). The number of vertical lines intersected by the drawn threshold line indicates the number of clusters. Thus, the dendrograms for both model depicts that the dataset is clustered into 4 clusters (Cluster A: No disease, Cluster B: Mild disease, Cluster C: Moderate disease, Cluster D: Severe disease) using Hierarchical clustering. Figures 3(a) and 3(b) depict the clustering results of estradiol against the oxidative stress marker, MDA, and protein carbonyl, respectively. Low levels of biomarkers (lesser than 6 and 1.25, respectively) have been identified as cluster A, irrespective of estradiol levels, implying that the individuals free of β-thalassemia disease do not have increased oxidative stress levels. On the other hand, extremely low levels of estradiol and very high MDA levels were appended as cluster D, depicting that these datasets correspond to severe disease. Figures 3(c) and 3(d) represent the cluster label identified in the plot of progesterone against the two oxidative stress markers. Similar to Figures 3(a) and 3(b), extremely low levels of the biomarkers represented cluster A, irrespective of the hormonal levels. For the male dataset, Figures 3(e) and 3(f) correspond to the plot of testosterone against MDA and protein carbonyl. The data samples corresponding to very low levels of MDA and protein carbonyl have been identified as Cluster A. On the other hand, extremely low testosterone levels, along with increased oxidative stress (high MDA and protein carbonyl), have been grouped as the most severe form of disease.

3.3. Classifier performance

The performances of the three classifiers have been assessed by generating the confusion matrix, and subsequently, the accuracy, specificity, and sensitivity scores have been evaluated. The 4×4 confusion matrixes were generated for all the classifiers, which estimated the performance of the classification model on a set of test data. Consequently, the accuracy, sensitivity, and specificity score were calculated. Sensitivity refers to the proportion of positive instances correctly classified as positive, while specificity refers to the proportion of negative instances correctly classified as negative. On the other hand, the proportion of instances correctly classified determines the accuracy. The performance of the classifiers for both datasets has been presented in Table 4. It can be observed that the Naive Bayes classifier gave the best accuracy, specificity, and sensitivity for the female dataset. However, the accuracy after KNN best achieved k cross-validation. In the case of the male dataset, SVM outperformed other classifiers.

3.4. Regression results

The neural network models were framed by optimizing the network architecture. Here, the transit function was found to be an efficient transfer function in all four cases. Optimum results are obtained with the configuration of several neurons in the hidden layer as 14 and 17 for MDA and protein carbonyl, respectively, for female patients. Similarly, the configuration for male patients was arrived as 10 and 17. The function logsig exhibited the lowest prediction efficiency with optimum RMSE and R2 (0.515 to 3.927 and 0.898 to 0.949). In contrast, the same performance indicators showed the optimal range of 0.092 to 0.809 for RMSE and 0.979 to 0.988 for R2, respectively, in the case of the transit function. The correlation coefficients for training, testing, and validation and an overall dataset for each model are presented in Figure 4 and Figure 5. The R-values for female MDA and protein carbonyl models are 0.995 and 0.989, inferring a high correlation for the overall dataset [Figure 4. (a), (b)]. Similarly, the correlation coefficients for male MDA and protein carbonyl are 0.992 and 0.994, respectively [Figure 5. (a), (b)]. The correlation coefficients for testing and validation of each model are compatible with that of the training dataset, implying the model's competency. Furthermore, the performance of the models for predicted and actual values was accessed using the analysis of variance (ANOVA) technique (Table 5). High Fisher's F-value (2042.035 to 6353.659) and low p-value (<0.001) of the overall dataset establish significant adequacy for each model. The mean square (MS) varied from 11.822 to 1069.430, showing the minimum value for the female protein carbonyl model and the maximum value for the male MDA model, respectively.

4. Discussion

The oxidative stress due to iron overload concerning the blood transfusion for β-thalassemia patients and the associated impact on gonadal and pituitary hormones were extensively studied through clinical survey and subsequent analysis of oxidative stress biomarkers, ferritin, and hormonal parameters. For both female and

male datasets, the FSH, LH, and gonadal hormone levels were significantly low in cases compared to control, implying that iron deposition-induced oxidative stress leads to the deranged secretion of these hormones and, therefore, the assessment of gonadal and other hormonal levels can be vital in determining the extent of severity among β-thalassemic patients. A structured protocol is formulated for detecting and categorizing acuteness of β-thalassemia considering the significant parameters. These parameters are collectively correlated to cluster the severity and classify after that, establishing a benchmark of the level of oxidative stress. The clustering results showed that individuals free of β-thalassemia disease do not have increased oxidative stress. On the other hand, extremely low levels of estradiol and testosterone in females and males, respectively, along with very high MDA levels, exhibited that these datasets correspond to individuals having severe disease. Furthermore, the prediction model proposed to passively assess oxidative stress biomarkers from the iron and various hormonal levels may avoid the complexity and high cost involved in the measurement of biomarkers. The proposed framework could, therefore, serve as an efficient real-life decision support system and benefit in the early diagnosis and prognosis of β-thalassemia disease.

5. Conclusion

In this study, the patients afflicted with β-thalassemia had enhanced serum ferritin levels, which thereby induces oxidative damage, such as increased lipid peroxidation (MDA) and protein oxidation (protein carbonyl). Consequently, such patients had decreased secretion of gonadal hormones (estradiol & progesterone in females and testosterone in male) and pituitary hormones (FS and LH). The study, therefore, validates the importance of early diagnosis and management of this endocrinopathy to give the patients a better quality of life. In this direction, the gonadal hormone levels, together with ferritin and oxidative stress biomarkers, could be beneficial in determining the extent of severity among such patients. Furthermore, ferritin, gonadal, and pituitary hormone levels could effectively determine MDA and protein carbonyl levels among such patients. In this context, the present study employs machine learning techniques, such as hierarchical clustering and classifiers. Naive Bayes, KNN, and SVM to assess the severity of the disease. The clustering results demonstrated that extremely low gonadal hormone levels and enhanced oxidative stress biomarkers corresponded to severe forms of disease. Among the classifiers, Naive Bayes depicted the best accuracy, sensitivity, and specificity for the female dataset, whereas SVM outperformed other classifiers in the case of the male dataset. Furthermore, the neural network efficiently predicted the oxidative stress biomarkers. The framework employed in the present study may be instrumental in the related area of medical science.

Acknowledgments: This work was supported by National medical college, Kolkata.

Author Contributions: All authors contributed equally to the writing of this paper. All authors read and approved the final manuscript.

Conflicts of Interest: "The authors declare no conflict of interest."

References

[1] Masala, G. L., Golosio, B., Cutzu, R., & Pola, R. (2013). A two-layered classifier based on the radial basis function for the screening of thalassaemia. *Computers in biology and medicine, 43*(11), 1724-1731.

[2] Galanello, R., & Origa, R. (2010). Beta-thalassemia orphanet. *Journal of Rare Diseases, 5*(11), 1172-75.

[3] Weatherall, D. J. (2001). Phenotype-genotype relationships in monogenic disease: lessons from the thalassaemias. *Nature reviews genetics, 2*(4), 245-255.

[4] Ribeil, J. A., Arlet, J. B., Dussiot, M., Cruz Moura, I., Courtois, G., & Hermine, O. (2013). Ineffective erythropoiesis in β-thalassemia. *The Scientific World Journal, 2013,* 394295.

[5] Basu, D., Sinha, R., Sahu, S., Malla, J., Chakravorty, N., & Ghosal, P. S. (2022). Identification of severity and passive measurement of oxidative stress biomarkers for β-thalassemia patients: K-means, random forest, XGBoost, decision tree, neural network based novel framework. *Advances in Redox Research, 5,* 100034.

[6] Fibach, E., & Rachmilewitz, E. A. (2017). Pathophysiology and treatment of patients with beta-thalassemia-an update. *F1000Research, 6.*

[7] Rivella, S. (2009). Ineffective erythropoiesis and thalassemias. *Current opinion in hematology, 16*(3), 187-194.

[8] Basu, D., Adhya, D. G., Sinha, R., & Chakravorty, N. (2021). Role of malonaldehyde as a surrogate biomarker for iron overload in the β-thalassemia patient: A systematic meta-analysis. *Advances in Redox Research, 3*, 100017.

[9] Trombetta, D., Gangemi, S., Saija, A., Minciullo, P. L., Cimino, F., Cristani, M., ... & Salpietro, C. D. (2006). Increased protein carbonyl groups in the serum of patients affected by thalassemia major. *Annals of hematology, 85*, 520-522.

[10] Srisukh, S., Ongphiphadhanakul, B., & Bunnag, P. (2016). Hypogonadism in thalassemia major patients. *Journal of clinical & translational endocrinology, 5*, 42-45.

[11] A Hagag, A., M Badraia, I., S Elfarargy, M., & M Abo El-enein, A. (2016). Gonadal hormones in adolescent females with β-thalassemia in relation to iron load. *Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders), 16*(2), 148-153.

[12] Roussou, P., Tsagarakis, N. J., Kountouras, D., Livadas, S., & Diamanti-Kandarakis, E. (2013). Beta-thalassemia major and female fertility: the role of iron and iron-induced oxidative stress. *Anemia, 2013.*

[13] Safarinejad, M. R. (2008). Evaluation of semen quality, endocrine profile and hypothalamus-pituitary-testis axis in male patients with homozygous β-thalassemia major. *The Journal of urology, 179*(6), 2327-2332.

[14] Jensen, C. E., ABDEL-GADIR, A., Cox, C., Tuck, S. M., & Wonke, B. (1996). Sperm concentrations and quality in β-thalassaemia major. *International journal of andrology, 19*(6), 362-364.

[15] Perera, D., Pizzey, A., Campbell, A., Katz, M., Porter, J., Petrou, M., ... & Chatterjee, R. (2002). Sperm DNA damage in potentially fertile homozygous β-thalassaemia patients with iron overload. *Human Reproduction, 17*(7), 1820-1825.

[16] Inati, A., Noureldine, M. A., Mansour, A., & Abbas, H. A. (2015). Endocrine and bone complications in β-thalassemia intermedia: current understanding and treatment. *BioMed research international*, 2015.

[17] Sinharay, M., Mitra, S., & Dasgupta, A. (2017). Effect of iron overload on gonadotrophins and organ sex steroids in pubertal thalassemia patients. *International Journal of Current Research and Review, 9*(23), 15-21.

[18] Abo-Elwafa, H. A., Hamid, S. A., Heshmat, M. M., & Ahmed, Z. S. (2017). Impact of ferritin load on gonadal reserve among regular transfused β-thalassemia. *Open Journal of Blood Diseases, 7*(02), 65-78.

[19] Mishra, N. K., & Celebi, M. E. (2016). An overview of melanoma detection in dermoscopy images using image processing and machine learning. *arXiv preprint arXiv:1601.07843.*

[20] Frid, A., Safra, E. J., Hazan, H., Lokey, L. L., Hilu, D., Manevitz, L., ... & Sapir, S. (2014, June). Computational diagnosis of Parkinson's disease directly from natural speech using machine learning techniques. In *2014 IEEE international conference on software science, technology and engineering (pp. 50-53)*. IEEE.

[21] Setsirichok, D., Piroonratana, T., Wongseree, W., Usavanarong, T., Paulkhaolarn, N., Kanjanakorn, C., ... & Chaiyaratana, N. (2012). Classification of complete blood count and haemoglobin typing data by a C4. 5 decision tree, a naï Bayes classifier and a multilayer perceptron for thalassaemia screening. *Biomedical Signal Processing and Control, 7*(2), 202-212.

[22] Jainontee, K., Lee, V. S., Prasitwattanaseree, S., & Grudpan, K. (2013). K-means Clustering and Hierarchical Cluster Analysis Coupled with Linear Discriminant Analysis to Classify Signals in Osmotic Fragility Test for Thalassemia Screening. *Journal of Natural Sciences, 12*. https://doi.org/10.12982/CMUJNS.2013.0013.

[23] Aszhari, F. R., Rustam, Z., Subroto, F., & Semendawai, A. S. (2020, March). Classification of thalassemia data using random forest algorithm. In *Journal of Physics: Conference Series* (Vol. 1490, No. 1, p. 012050). IOP Publishing.

[24] Upadhyay, A. (2012). β-thalassemia major and minor classification using artificial neural network. *International Journal of Computers and Applications, 13*, 14-17.

[25] Tayefi, M., Esmaeili, H., Karimian, M. S., Zadeh, A. A., Ebrahimi, M., Safarian, M., ... & Ghayour-Mobarhan, M. (2017). The application of a decision tree to establish the parameters associated with hypertension. *Computer methods and programs in biomedicine, 139*, 83-91.

[26] Abedi, R., Costache, R., Shafizadeh-Moghadam, H., & Pham, Q. B. (2022). Flash-flood susceptibility mapping based on XGBoost, random forest and boosted regression trees. *Geocarto International, 37*(19), 5479-5496.

[27] Piroonratana, T., Wongseree, W., Assawamakin, A., Paulkhaolarn, N., Kanjanakorn, C., Sirikong, M., ... & Chaiyaratana, N. (2009). Classification of haemoglobin typing chromatograms by neural networks and decision trees for thalassaemia screening. *Chemometrics and Intelligent Laboratory Systems, 99*(2), 101-110.

[28] Ellethy, H., Chandra, S. S., & Nasrallah, F. A. (2021). The detection of mild traumatic brain injury in paediatrics using artificial neural networks. *Computers in Biology and Medicine, 135*, 104614.

[29] Chen, T. S., Tsai, T. H., Chen, Y. T., Lin, C. C., Chen, R. C., Li, S. Y., & Chen, H. Y. (2005, December). A combined K-means and hierarchical clustering method for improving the clustering efficiency of microarray. In *2005 International symposium on intelligent signal processing and communication systems* (pp. 405-408). IEEE.

[30] Python Software Foundation, (2020).

[31] Suguna, N., & Thanushkodi, K. (2010). An improved k-nearest neighbor classification using genetic algorithm. *International Journal of Computer Science Issues, 7*(2), 18-21.

[32] Chandel, K., Kunwar, V., Sabitha, S., Choudhury, T., & Mukherjee, S. (2016). A comparative study on thyroid disease detection using K-nearest neighbor and Naive Bayes classification techniques. *CSI transactions on ICT, 4*, 313-319.

[33] Vembandasamy, K., Sasipriya, R., & Deepa, E. (2015). Heart diseases detection using Naive Bayes algorithm. *International Journal of Innovative Science, Engineering & Technology, 2*(9), 441-444.

[34] Amendolia, S. R., Cossu, G., Ganadu, M. L., Golosio, B., Masala, G. L., & Mura, G. M. (2003). A comparative study of k-nearest neighbour, support vector machine and multi-layer perceptron for thalassemia screening. *Chemometrics and Intelligent Laboratory Systems, 69*(1-2), 13-20.

[35] Murugesan, M., & Elankeerthana, R. (2018). Support vector machine the most fruitful algorithm for prognosticating heart disorder. *International Journal of Engineering & Technology 7*(2.26), 48-52.

[36] Gupta, A. K., Ghosal, P. S., & Srivastava, S. K. (2017). Modeling and optimization of defluoridation by calcined Ca-Al-(NO 3)-LDH using response surface methodology and artificial neural network combined with experimental design. *Journal of Hazardous, Toxic, and Radioactive Waste, 21*(3), 04016024.

[37] Ghosal, P. S., & Gupta, A. K. (2018). Sorptive equilibrium profile of fluoride onto aluminum olivine [(Fe x Mg1- x) 2SiO4] composite (AOC): Physicochemical insights and isotherm modeling by non-linear least squares regression and a novel neural-network-based method. *Journal of Environmental Science and Health, Part A, 53*(12), 1102-1114.

[38] Singer, S. T., Vichinsky, E. P., Gildengorin, G., van Disseldorp, J., Rosen, M., & Cedars, M. I. (2011). Reproductive capacity in iron overloaded women with thalassemia major. Blood, *The Journal of the American Society of Hematology, 118*(10), 2878-2881.