Australasian Physical & Engineering Sciences in Medicine (2019) 42:733–743
https://doi.org/10.1007/s13246-019-00778-x

SCIENTIFIC PAPER

An efficient heart murmur recognition and cardiovascular disorders classification system

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Received: 18 March 2019 / Accepted: 24 June 2019 / Published online: 16 July 2019

Abstract

The problem addressed in this work is the detection of a heart murmur and the classification of the associated cardiovascular disorder based on the heart sound signal. For this purpose, a dataset of Phonocardiogram (PCG) signals is acquired using baseline conditions. The dataset is acquired from 283 volunteers using Littman 3200 electronic stethoscope for a normal and four different types of heart murmurs. The samples are labelled and validated through echocardiography test of each participating volunteer. For feature extraction, normalized average Shannon energy with time-domain characteristics of heart sound signal is exploited to segment the PCG signal into its components. To improve the quality of the features, in contrast to the previous methods, all systole and diastole intervals are utilized to extract 50 Mel-Frequency Cepstrum Coefficients (MFCC) based features. Then, the iterative backward elimination method is used to identify and remove the redundant features to reduce the complexity in order to conceive a computationally tractable system. An MFCC feature vector of dimension 26 is selected for training seven different types of Support Vector Machine (SVM) and K-Nearest Neighbors (KNN) based classifiers for detection and classification of cardiovascular disorders. Fivefold cross-validation and 20% data holdout validation schemes are used for testing the classifiers. Classification accuracy of 92.6% is achieved using selected features and medium Gaussian SVM classifier. The learning curves show a good bias-variance trade-off indicating a well-fitted and generalized model for making future predictions.

Keywords Heart murmur detection · Cardiovascular disorders classification · MFCC · SVM · KNN

Introduction

Heart disease commonly referred to as Cardiovascular Disorder (CVD) is one of the leading global causes of mortalities. It accounts for 17.3 million deaths per year, an amount that is estimated to rise to more than 23.6 million by 2030 [1]. According to the latest World Health Organization (WHO) report [1], CVD alone causes nearly 0.12 million fatalities in Pakistan, which amounts to 9.87% of the total death toll. Early detection of heart disease is therefore vital and is carried out through echocardiogram test or perceiving heart murmur by a physician during auscultation. Auscultation is a process of listening to heart sound signal and perceiving its components using a stethoscope. It remains the most popular technique to identify the possible occurrence of CVD due to the minimal equipment requirement and low cost in comparison to the echocardiogram test.

Heart murmurs are the unusual audible sounds produced during a heartbeat cycle. While murmurs could be harmless (innocent), they are generally an indication of the underlying CVD. Therefore, identification of heart murmur at an initial stage of its onset is very crucial as it is an indication of the heart problems like heart valves leakage, narrowing of valves, intra-cardiac shunt or valvular lesions etc. [2, 3] which can lead to fatality [4]. However, primary care physicians need extensive experience and training for auscultation to identify the heart murmurs during a routine checkup. With the reported accuracy rate of 20–40% on the auscultation process [2], CVDs are generally neither timely diagnosed nor investigated mainly due to the under-diagnosis by the physicians and unawareness in heart disease patients of the

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CVD symptoms. Early diagnosis of CVDs can be achieved with the help of Computer Aided Detection (CAD) systems. With the rapid advancement in imaging techniques [5] along with the availability of low-cost wearable sensors [6] and excessive computational power [7], CAD systems can play a decisive role in early diagnosis.

A considerable amount of work has been done towards the identification and classification of heart murmurs from heart sound signals. These studies are based on the analysis of Phonocardiogram (PCG) signals, i.e., a digital heart sound signal recorded through an electronic stethoscope, and usually involves three steps. The first step is pre-processing of the PCG signal which involves denoising and segmentation. PCG signal denoising is generally achieved through the utilization of suitable filters [2, 8] and wavelet transforms [9–11]. For PCG signal segmentation, several techniques have been proposed based on Hidden Markov Model [2, 12, 13], Shannon and Average Shannon energy [9, 11, 14–16], Wavelet decomposition [17], Autocorrelation function [3], Hilbert phase envelope [18], Ensemble empirical mode decomposition [19], Autoregressive power spectral density function [20] and fixed windowed and moving windowed Hilbert transform [21, 22]. The second step involves the feature extraction from the segmented PCG signal. Techniques reported for the feature extraction are based on the Mel-Frequency Cepstrum Coefficients (MFCCs) [2, 3, 12, 13, 19], spectral [23, 24], statistical [9, 16, 20, 21, 25] and temporal features. Finally, the classification of PCG signals based on the extracted features is generally performed by utilizing machine learning tools which include Artificial Neural Networks (ANNs), Support Vector Machine (SVM) [2, 11, 14, 26], and K-Nearest Neighbors (KNNs) [23, 25].

The previous work for Heart Murmur Recognition (HMR) generally employs an extensive feature set for classification and is based on a minimal dataset with mostly two classes (normal and abnormal PCG signals). As a result, such systems are not only computationally expensive but also over-fitted for a particular dataset and therefore, can neither provide the best diagnostic results efficiently nor classify different heart murmurs. In this paper, we address the aforementioned problems and propose an efficient machine learning based HMR and CVDs classification system. The major contributions of this work are as follows:

- A dataset of five types of PCG signals (1 normal and 4 abnormal) is collected in hospital environment from multiple volunteered CVD patients. The dataset is labelled through auscultation and validated through echocardiogram test of each volunteer.
- A computationally simple method is presented with reduced complexity due to the extraction and utilization of improved small feature set selected through detailed analysis.
- A robust system is proposed which can detect and classify four different CVDs pertaining to different heart murmurs with substantially high accuracy due to training on a newly collected comprehensive dataset.

The remainder of the paper is organized as follows: Sect. "Heart physiology and PCG signal components" details the heart physiology and PCG signal components. Section "Methodology" presents the proposed system for HMR and CVDs classification. Experimental results are presented in Sect. "Result" and discussed in Sect. "Discussion". Finally, Sect. Conclusion presents the conclusion and future work.

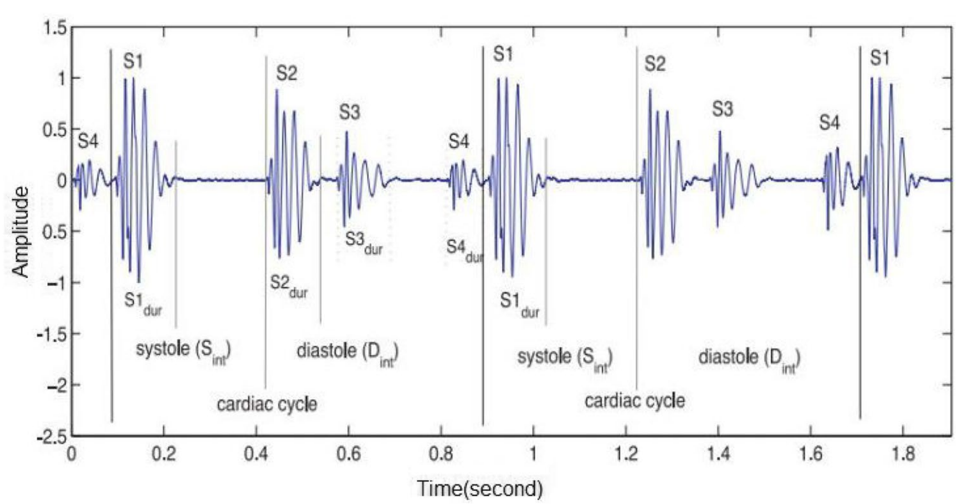

Fig. 1 Structure of an abnormal human heart sound signal

Heart physiology and PCG signal components

The human heart produces a sound signal due to the closure of heart valves. The PCG signal is made up of two intervals: Systole and Diastole, and up to four components S1, S2, S3 and S4. Figure 1 depicts the structure of an abnormal heart sound signal. Heart valves aid in pumping blood through the heart four chambers. Blood flow from the upper two chambers (left atria and right atria) to lower two chambers (left ventricle and right ventricle) is controlled through the tricuspid and mitral valves. The closing of these two valves results in the S1 component and is best heard in the mitral area of the heart. The S2 component is best heard over the aortic area and results due to the closing of aortic and pulmonary valves which controls the blood flow out of the ventricles. Diastole and Systole intervals correspond to the hearts blood filling and leaving stages, respectively.

While the normal heart PCG signal constitutes of S1 and S2 component only, the presence of additional components S3 and S4, referred to as murmurs, is an indication of an abnormal heart condition [2, 4, 8, 9]. Murmurs are classified into systolic and diastolic murmurs based upon their occurrence in the heart sound signal. Systolic murmurs occur between S1 and S2 components and are caused by Aortic Stenosis, Mitral Regurgitation or Ventricular Septal Defect [8, 27]. Diastolic murmurs occur after S2 and are therefore associated with ventricular relaxation and filling. Aortic Regurgitation or Mitral Stenosis cause this type of murmur. Therefore, segmentation, analysis and classification of PCG signals can lead to the identification of particular heart murmur and underlying CVD.

Methodology

The proposed system for HMR and CVDs classification is depicted in Figure 2. Details of each processing step are mentioned next.

Data acquisition

One of the main challenges in studies related to the HMR is the availability of heart sound signals. While there are a number of PCG signal databases available, as mentioned in Table 1, they are either; (i) limited to very few samples, (ii) close sourced and not globally approved, (iii) not captured in clinical environment, or (iv) limited to normal and abnormal PCG signals only (without murmur classification). Therefore, a new dataset has been acquired to resolve these previous issues. The heart sound samples were captured from the patients referred to the cardiology department of Ayub Teaching Hospital, Abbottabad, Pakistan. A total of

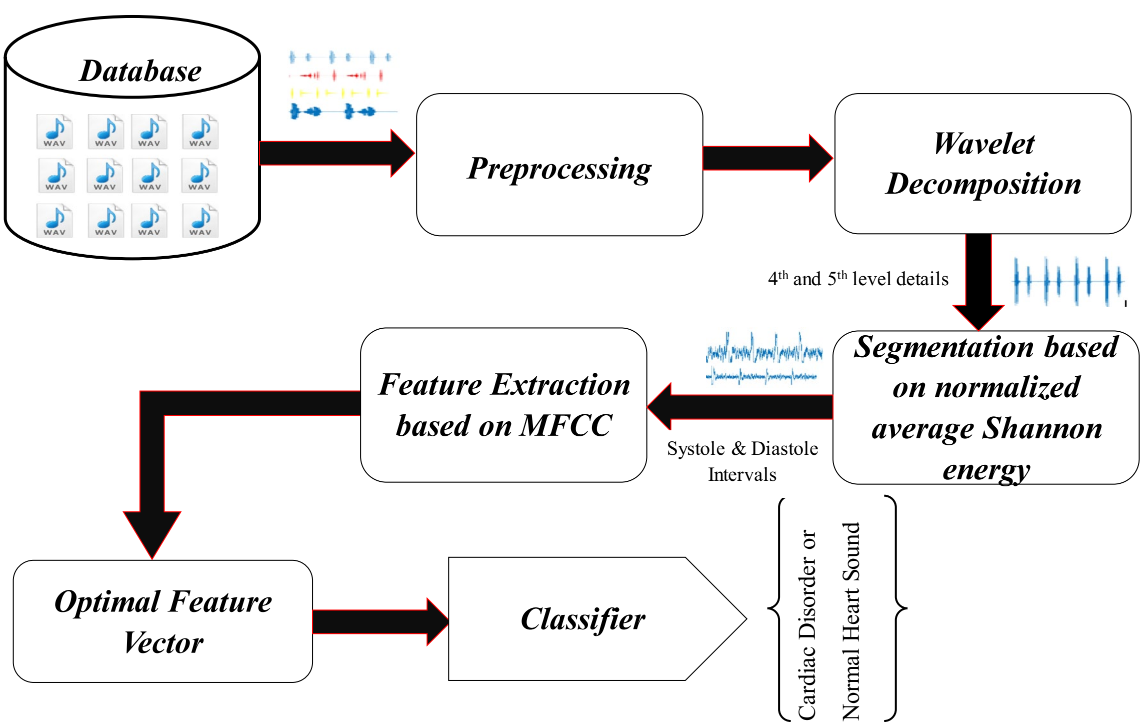

Fig. 2 Proposed system's framework

Table 1 Limitations of various PCG datasets

Dataset name	PCG types	Limitations
Daniel Mason: listening to the heart [28]	Normal and murmurs	Close sourced, not globally approved
eGeneral Medical [29]	Normal, murmurs, clicks, and splits	Limited (64 murmur samples and 01 normal sample), not globally approved
Michigan [30]	Normal and systolic murmurs only	Limited (45 murmur samples and 02 normal samples), not globally approved
Bioscience [31]	Normal and murmurs	Limited (25 murmur samples and 01 normal sample), not globally approved
PASCAL classifying heart sounds challenge [32]	Normal, abnormal, extra heart sound, and artifacts	Murmurs are not labelled, not globally approved
Cardionics Inc [33]	Normal and murmurs	Close sourced, not globally approved
PhysioNet challenge [34]	Normal and abnormal	Murmurs are not labelled

Table 2 Characteristics of PCG signal components

PCG signal component	Frequency (Hz)	Time (ms)
S1	30–100	50–100
S2	> 100	25–50
S3	20–25	120–150
S4	< 30	90

283 sound samples were collected from 256 males and 27 females with age ranging from 25 to 48 years. An electronic stethoscope (model 3M Littman 3200) was utilized and connected with a computer for the acquisition of sound signal in .wav format with a sampling frequency of 11,025 Hz and 16-bit resolution.

All recordings were taken in the hospital environment and under the supervision of an expert physician from the apex, aortic, tricuspid and pulmonic area of the human heart. The captured dataset contains 175 normal heart sound samples and 108 abnormal heart sound samples having a murmur. Among abnormal samples, 28 samples are of Aortic Stenosis (AS), 26 for Mitral Stenosis (MS), 27 for Aortic Regurgitation (AR), and 27 for Mitral Regurgitation (MR). Labelling of the samples is done by an expert cardiologist through auscultation which is further validated through echocardiography test of each participating subject.

Preprocessing

During PCG signal acquisition, some unwanted noise is coupled with the original heart sound signal which needs to be filtered out. The noise generally includes Humming and Ambient (background) noise which are mainly added due to the clinical environment, capturing device imperfections and other operating biomedical devices. Humming noise is low-frequency in nature and can be considered one of the main reasons for heart murmurs not being detected during auscultation. It can mask low-frequency PCG signal components

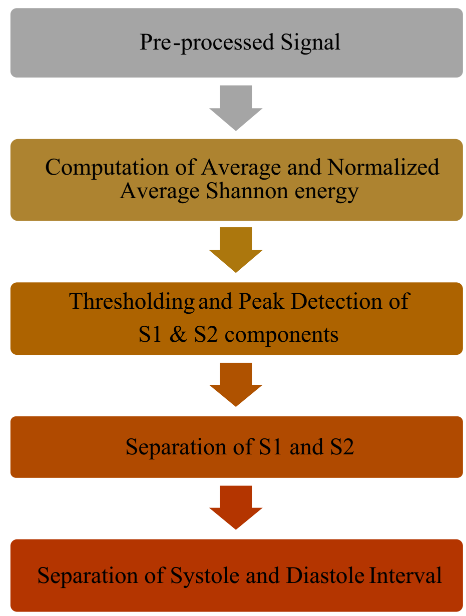

Fig. 3 Segmentation algorithm

S3 and S4, which are the indicators of a heart murmur. On the other hand, ambient noise has high frequency and can, therefore, make the detection and segmentation of the high-frequency S1 and S2 PCG signal components (refer to Table 2) quite challenging. Therefore, signal denoising is performed in the pre-processing stage.

Wiener filter and wavelet denoising techniques are well known to yield effective results to estimate and remove Ambient noise [2, 8–11]. In the proposed framework, wavelet decomposition and reconstruction is applied after normalizing and down-sampling the captured PCG signals. The mother wavelet db6 is chosen from Daubechies wavelet family due to its resemblance in shape to S1 and S2 PCG signal components [35]. For reconstruction, hard thresholding is performed and 4th and 5th level details were chosen

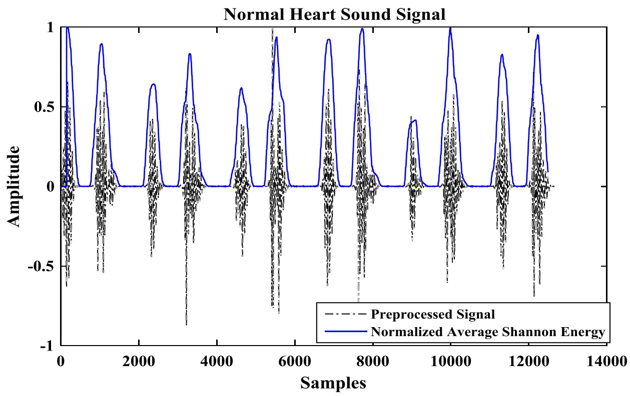

Fig. 4 Normalized average Shannon energy

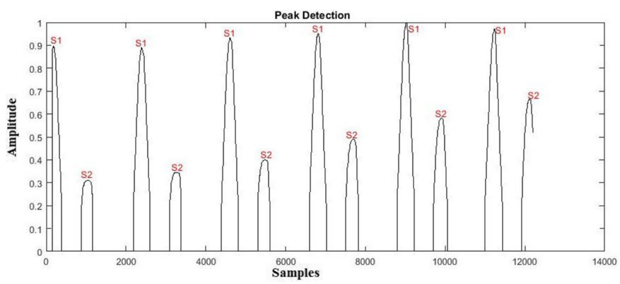

Fig. 5 Peak detection of S1 and S2

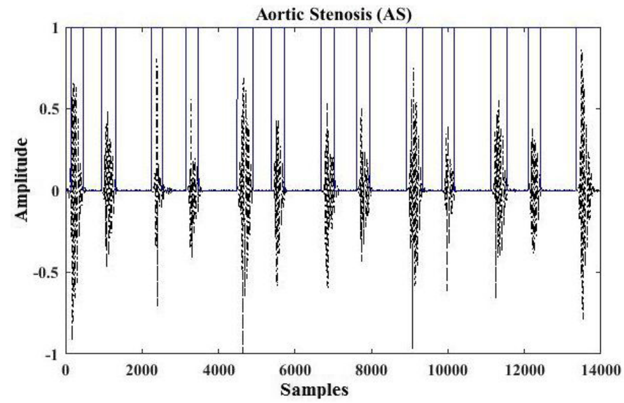

Fig. 6 Isolation of S1 and S2 peaks

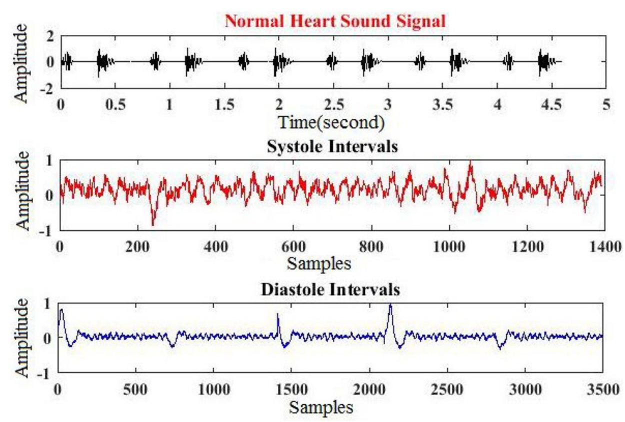

Fig. 7 Isolation of systole and diastole intervals of normal heart sound signal

due to the maximum power of S1 and S2 components in these levels.

Segmentation

The segmentation process in proposed HMR and CVDs classification system is shown in Figure 3. The pre-processed PCG signal is segmented into its components by extracting the envelope and using the time-domain characteristics of the PCG signal. For envelope computation, an average and normalized average Shannon energy is computed using:

$$E_s = \frac{-1}{L} \sum_{j=1}^{L} x_p^2(j) * \log(x_p^2(j)) \tag{1}$$

$$P = E_s - \bar{E}_s \tag{2}$$

where L is the length of window, x_p is the reconstructed signal from previous stage.

The Shannon energy boosts the frequency regions where S1 and S2 components are located and therefore, is the most commonly used envelope based segmentation technique. Figure 4 shows the envelope extraction through normalized average Shannon energy. Amplitude thresholding is then applied to Shannon entropy values to detect and identify the peaks of S1 and S2. Specific amplitude thresholding with an amplitude of 0.2 is utilized in this work due to its better results in comparison to the adaptive amplitude thresholding. The detected peaks are identified as S1 and S2 by inspecting the amplitudes of two adjacent peaks and labelling the large amplitude peaks as S1 and small amplitude peaks as S2 as depicted in Fig. 5. After labelling, segmentation of PCG signal into its components is done by exploiting the well-established time-domain characteristics of the heart sound signal. The starting and ending points of the labelled S1 and S2 peaks are identified and a single period of PCG signal is extracted. Then based on a criterion that the width of the S1 component is generally larger than the S2 component, S1 and S2 components are isolated as shown in Fig. 6. Further, diastole and systole intervals are segmented based on the fact that systole interval is always shorter in duration in comparison to the diastole interval and is preceded and followed by S1 and S2 components, respectively. Figures 7 and 8 show the systole and diastole intervals of each case considered in this work.

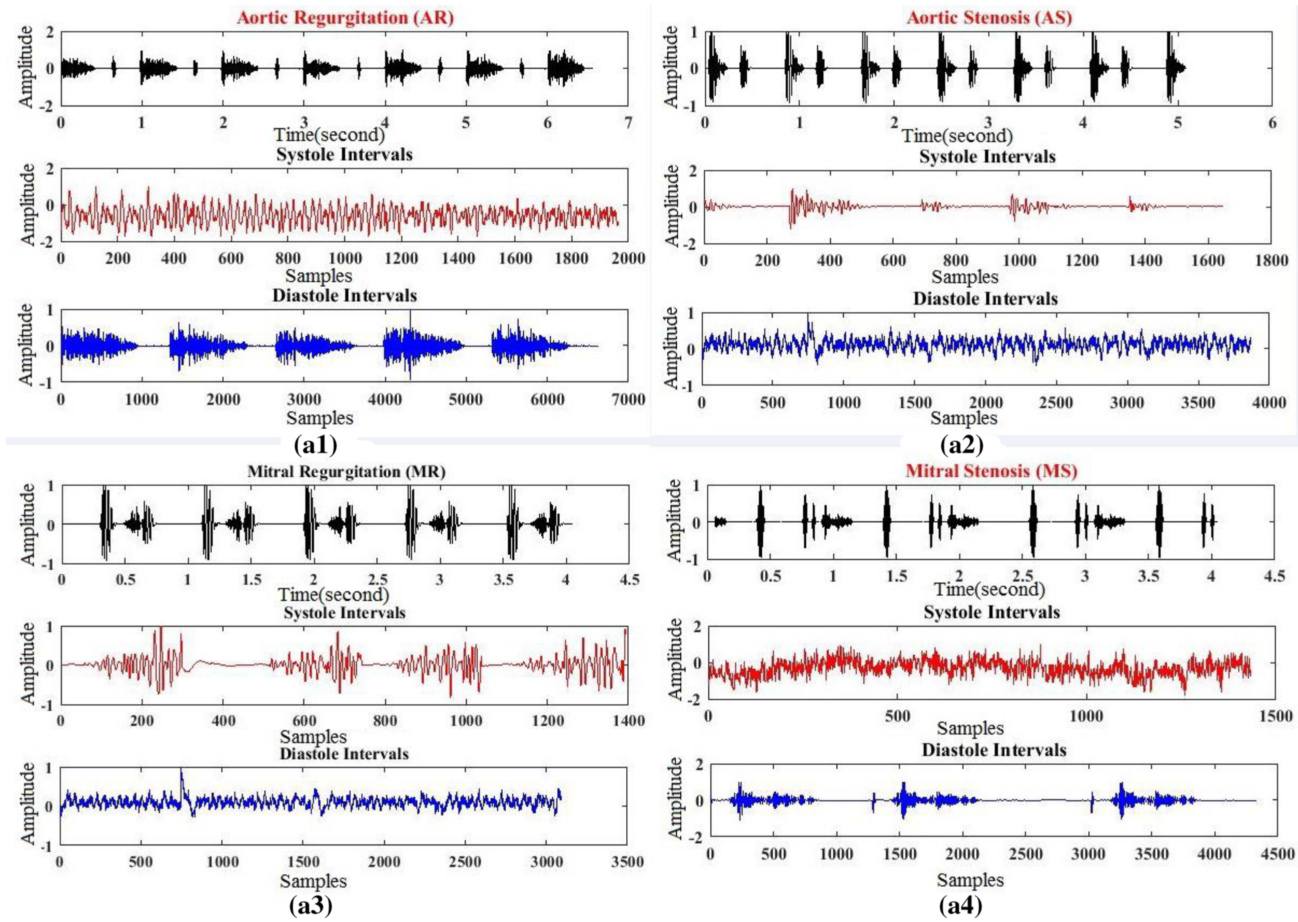

Fig. 8 Isolation of systole and diastole intervals of heart murmur: **a1** AR signal, and its systole, diastole intervals, **a2** AS signal, and its systole, diastole intervals, **a3** MR signal, and its systole, diastole intervals, **a4** MS signal, and its systole, diastole intervals

Feature extraction and classification

In order to detect and classify the occurrence of heart murmurs, 50 Mel-Frequency Cepstrum Coefficients (MFCCs) [13] based features are extracted by using MFCC order of 45 with a hamming window size of 25 ms. While most of the existing work considers only a single systole and diastole interval concatenated for feature extraction, the proposed method utilizes all segmented systole and diastole intervals from the PCG signal for improving the quality of extracted feature vector. Further, techniques reported for feature extraction based on MFCCs [2, 3, 12, 13, 19] mostly utilize extensive feature set including spectral as well as temporal features. However, most of these features are generally redundant and contribute towards increasing the computational cost without increasing the efficiency of the classifier. In the proposed system, optimal MFCC feature vector dimension is 26 which is selected after removing the redundant features through iterative backward elimination method. In this method, each feature is checked individually for its effect on the accuracy of the classifier and only those features are retained which have a substantial effect on the classification accuracy upon removal.

For classification, Support Vector Machine (SVM) and K-Nearest Neighbors (KNN) classifiers are utilized which are trained on the selected extracted features. The SVM is a kernel-based classifier and operates on the principle of margin maximization. It performs multi-class classification by constructing a hyperplane to divide data into multiple groups with the largest possible separation. The KNN is a distance metric based classifier and operates on the principle of similarity. KNN algorithm searches for K nearest neighbors of the query data using the predefined distance metric and assigns a class based on a voting scheme.

Results

Seven different types of SVM and KNN based classifiers were trained and tested using the extracted features to analyze the accuracy of the proposed HMR and CVDs classification system. For using SVM, a Radial Basis Function (RBF) kernel based multi-class Error-Correcting Output Codes (ECOC) model with One vs. One strategy was created. Different kernel functions were also tested for the optimization of the proposed system. This technique constructs n(n−1)/2 SVM models by labelling each pair of separate classes with positive and negative. n is the number of distinct classes, which in our case is 5. KNN is used with one nearest neighbor and Euclidean distance metric with maximum voting strategy.

For training and testing the selected classifier models for optimal feature vector dimension, two validation schemes namely N-fold cross-validation and N% data holdout are used, which are based upon the division of data samples for training and testing phases. We have opted fivefold cross-validation in which the data samples are randomly partitioned into five equal sets and classifier is trained on all sets leaving one set out on which testing is performed. The same procedure is applied for each set and resultant accuracy is collectively calculated for the whole set. For holdout validation scheme, 20% data holdout is employed and the test-train ratio of 1:4 is selected where 80% of the data is randomly chosen for training and the remaining 20% is used for testing.

Table 3 presents the classification accuracy of the tested classifiers with different dimensions of selected MFCC feature vector using a fivefold cross-validation scheme. Results indicate that, of all the tested classifiers, medium Gaussian SVM has the highest accuracy of 92.6% with MFCC feature vector dimension of 26 indicating the presence of redundant features. Accuracy starts decreasing if the feature vector dimension is reduced below 26. Similar observations can be generally inferred from the Table 4, which tabulates the classification accuracy based on the 20% data holdout validation technique.

Table 3 Classification accuracy (%) with different MFCC feature vector dimension using fivefold cross-validation

Feature vector dimension	Accuracy of the classifier						
	Fine KNN	Medium KNN	Weighted KNN	Linear SVM	Cubic SVM	Quadratic SVM	Medium Guassian SVM
50	87.6	82.7	85.5	88.0	88.3	89.4	89.4
46	86.9	82.0	85.9	88.0	86.9	89.0	89.4
40	87.3	82.7	86.9	88.3	86.9	88.7	89.8
36	86.2	82.7	86.6	89.0	87.6	88.3	90.1
30	86.2	83.0	86.6	89.0	87.6	88.3	90.8
26	87.3	83.7	88.3	87.6	87.6	89.0	92.6
20	84.1	82.0	86.2	89.4	87.3	89.0	91.2
15	82.0	80.6	83.0	89.4	85.5	87.6	89.0
10	82.3	80.6	85.9	86.9	85.5	88.3	86.6

Table 4 Classification accuracy (%) with different MFCC feature vector dimension using 20% data holdout

Feature vector dimension	Accuracy of the classifier						
	Fine KNN	Medium KNN	Weighted KNN	Linear SVM	Cubic SVM	Quadratic SVM	Medium Guassian SVM
50	80.0	80.0	82.5	85.0	90.0	90.0	85.0
46	75.0	75.0	82.5	85.0	85.0	87.5	82.5
40	82.5	80.0	82.5	80.0	85.0	85.0	85.0
36	77.5	77.5	85.0	80.0	87.5	82.5	85.0
30	75.0	82.5	87.5	72.5	80.0	80.0	82.5
26	77.5	82.5	85.0	85.0	80.0	82.5	85.0
20	75.7	80.4	83.9	84.5	87.5	85.7	84.5
15	72.1	78.6	84.7	84.5	82.1	83.9	84.7
10	75.7	80.4	84.7	83.9	87.5	87.5	83.7

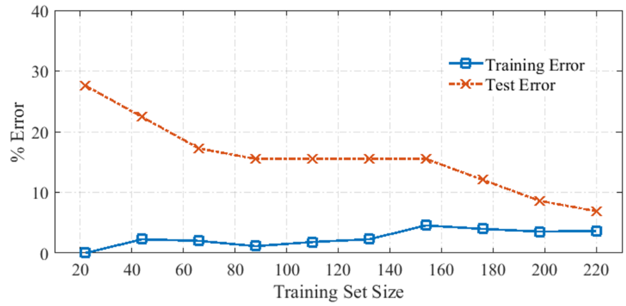

Fig. 9 Training and test error of the medium Guassian SVM

Table 5 Confusion matrix with an average accuracy of 88.3% using weighted KNN

Cardiac Disorder	N	AS	MS	AR	MR
N	175 100%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
AS	8 28.6%	17 60.7%	0 0.0%	0 0.0%	3 10.7%
MS	1 3.8%	1 3.8%	22 84.6%	1 3.8%	1 3.8%
AR	1 3.7%	0 0.0%	5 18.5%	21 77.8%	0 0.0%
MR	4 14.8%	8 29.6%	0 0.0%	0 0.0%	15 55.6%

To assess the trained model behavior, the training and test learning curves for medium Gaussian SVM are computed and presented in Fig. 9. It can be observed that it is a well-fit model. The low bias reflected by the low training error indicates that the model is well-fitted to the training data. Moreover, the low test error and variance indicates that the model is well generalized and does not has the problem of over-fitting.

Discussion

Three types of KNN classifiers namely Fine KNN, Medium KNN and Weighted KNN are tested on different dimensions of MFCC feature vector. As indicated in Table 3, among these tested classifiers, the highest classification accuracy of 88.3% is achieved by using Weighted KNN with MFCC feature vector dimension of 26 using fivefold cross-validation scheme. Table 5 presents the confusion matrix of Weighted KNN classifier for five different classes. While the diagonal entries represent the percentage of accurately detected heart disease, the off-diagonal entries denote the number and percentage of CVD that is confused by the classifier with other types. It can be observed that normal heart sound is classified without any error thereby avoiding the error of detecting heart abnormality in a healthy person. Figure 10 illustrates the average efficiency of the selected KNN classifiers (with optimal MFCCs feature vector dimension of 26 and fivefold cross-validation scheme) for each CVD and normal heart sound. The normal heart sound has the highest classification accuracy for each classifier.

For SVM classifier, four types are utilized which include Linear SVM, Cubic SVM, Quadratic SVM and medium Gaussian SVM. As done before for KNN, these classifiers are tested on different MFCC feature vector sizes and accuracy of the classifier is observed through fivefold cross-validation scheme. The medium Gaussian SVM with optimal 26 selected MFCC features produced the highest accuracy results of 92.6% amongst all. Table 6 shows the confusion

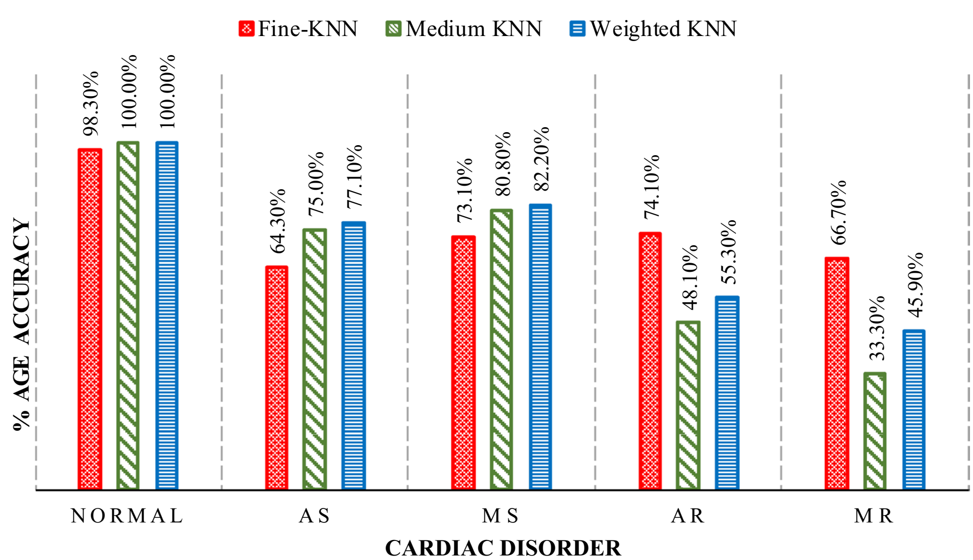

Fig. 10 Average accuracy of KNN classifiers for CVD classification

Table 6 Confusion matrix with an average accuracy of 92.6% using medium Gaussian SVM

Cardiac Disorder	N	AS	MS	AR	MR
N	174 99.4%	0 0.0%	1 0.6%	0 0.0%	0 0.0%
AS	0 0.0%	25 89.3%	0 0.0%	0 0.0%	3 10.7%
MS	1 3.8%	0 0.0%	22 84.6%	1 3.8%	2 7.7%
AR	0 0.0%	0 0.0%	5 18.5%	22 81.5%	0 0.0%
MR	2 7.4%	6 22.2%	0 0.0%	0 0.0%	19 70.4%

matrix of cardiac classification with an average accuracy of 92.6% using medium Gaussian SVM classifier with 26 MFCCs. Figure 11 depicts the average efficiency of the selected SVM classifiers (with 26 dimensions of MFCCs and fivefold validation scheme) for each cardiac disorder as well as normal heart sound. As observed before, the normal heart sound has the highest classification accuracy for each tested classifier. This is mainly due to the distinct and unique features in normal heart PCG signal in which systole and diastole intervals are generally silent. Figure 12 illustrates the overall efficiency comparison of SVM and KNN classifiers on both the fivefold cross-validation scheme and 20% holdout validation scheme with 26 MFCCs. The results reveal that SVM performs better for both fivefolds and 20% holdout validation schemes with an average accuracy of 92.6% and 90.00%, respectively.

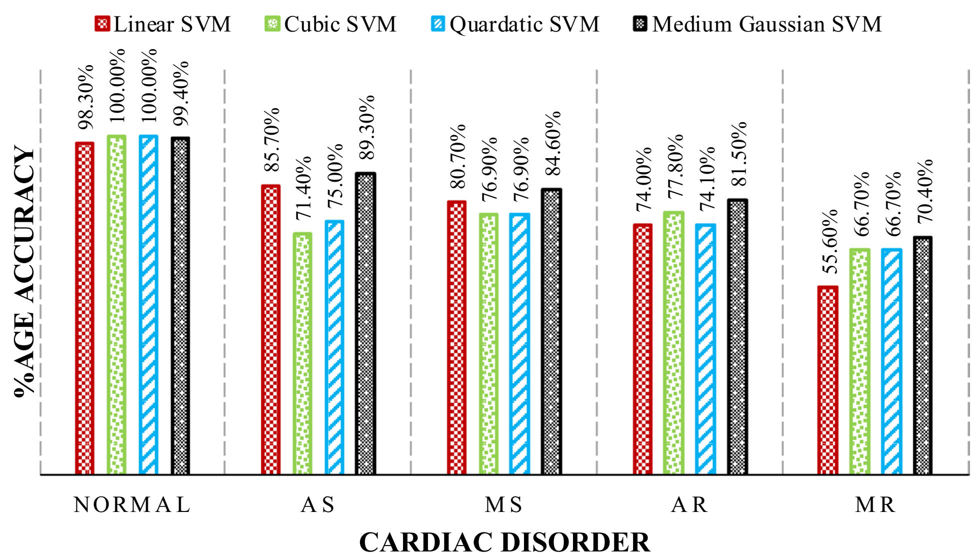

Fig. 11 Average accuracy of SVM classifiers for CVD classification

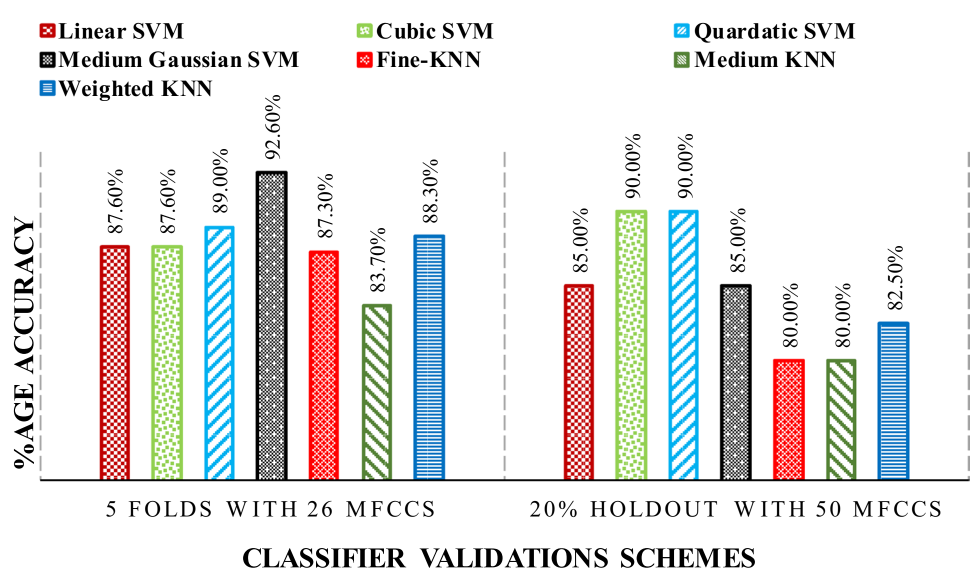

Fig. 12 Classification accuracy comparison of SVM and KNN classifiers

Table 7 A comparison between the proposed method and related work

Classification method [dataset name (if any)]	CVD types	Average (%) accuracy	Feature vector dimension
RBF, MLP, SVM [2] (Daniel Mason: listening to the heart)	Normal, Ventricular Septal Defect (VSD), MR, AR, MS, AS, AR+AS, MR+MS, Mitral Valve Prolapse (MVP)	RBF = 84.4 MLP = 83.1 SVM = 85.6	39 MFCC features
k-mean [3]	Ejection systolic and pan-systolic heart murmur	61.1	167 Temporal and spectral features
Fuzzy logic controller [9]	Normal, MS, AS, AR	80	04 Temporal and spectral features
SVM [11]	Normal and abnormal	83.5	Multiple temporal features
SVM [14] (PASCAL & PhysioNet challenge)	Normal and abnormal	Dataset A = 67% Dataset B = 83% Dataset C = 88%	Spectrogram features of 10 x 10 core tensor size
ANN [20] (Michigan)	09 Types of cardiac abnormalities	88.89	07 Temporal and spectral features
Growing time SVM [23]	Normal and AS	88	Not informed
Naïve Bayes, KNN, SVM [26] (PASCAL)	Normal and heart murmur	Dataset A = 78% Dataset B = 80%	12 Temporal features
Proposed	Normal, MS, MR, AS, AR	SVM = 92.6% , KNN = 88.3%	26 MFCC features

The comparison of the proposed method with recent state-of-the-art HMR and CVD classification methods reported in the literature is given in Table 7. However, it must be noted here that a one to one comparison is not possible as the dataset used for each work is different. It can be observed that the proposed method outperforms previous schemes and achieves the highest classification accuracy for HMR and CVDs classification. Further, our method is computationally simple being based on a small feature vector in comparison to the similar MFCC based methods [2, 3, 14]. It is pertinent to mention here that while the methods in [9, 20, 26] use less number of features than ours, they are based on a dataset of insignificant sizes.

Conclusion

In this paper, an efficient heart murmur recognition and cardiovascular disorders classification system is proposed. The method can efficiently detect the heart murmur and can classify up-to four different CVDs with an average accuracy of 92.6%. The limitations of small PCG datasets and computationally expensive schemes in previously proposed methods are addressed by capturing a novel PCG dataset of 283 samples and utilizing computationally tractable PCG segmentation scheme along with an optimal feature vector size of reduced dimension for training classifiers. As the PCG dataset is captured during real-time checkups in a hospital environment, the well-fitted classifier model indicates that the proposed system can be utilized to aid physicians in detecting and classifying heart murmurs. Future work will be aimed towards predicting the stage of cardiac disorder or heart failure on the basis of heart murmur. Further, the PCG dataset size will be enlarged by incorporating more murmur types and samples.

Acknowledgements We would like to thank Dr. Imran, Head of Cardiology Department, Ayub Teaching Hospital, Abottabad, Pakistan, who allowed the utilization of hospital room. We are also thankful to Dr. Shahid Khattak, Chairman Electrical Engineering Department Comsats institute of information technology, Abbottabad for providing us an Electronic Stethoscope for dataset capturing.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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