ASSESSMENT OF THE REACTIVE HYPERAEMIA THROUGH QUANTIFICATION OF COLOUR CHANGES USING PIXEL ANALYSIS SOFTWARE

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Abstract:
A sound cardiovascular system is essential for human health. There are many effective tests to measure cardiac performance, but no matching test exists to detect vascular reactivity. Hence the acute need for a sensitive, non-invasive, cost-effective screening test. The present study purports such a method of assessing variation in blood flow by quantifying colour changes visible in palm secondary to reactive hyperaemia for detection of presence of vascular lesions.
Colour changes in palms of test and control hands were recorded in healthy young volunteers (5 males, 5 females) by taking a series of photographs at 5 seconds interval in pre, during and post occlusion periods. Sphygmomanometer was used to produce arterial occlusion in test hand. The colour changes were analysed for red pixels by indigenously developed colour analysis software "Chitradeepam". Mean of values for each hand in above-mentioned periods were calculated for all subjects and corresponding values of test and control hands were compared using student's t test.
No difference was observed in baseline values of both hands. During occlusion, there was significant decrease in test hand colour in females compared to their control hand but not in males. Post occlusion, significant colour increase was observed in test hands of males compared to control. A similar trend, though statistically not significant was seen in females.
This technique can be useful in determining normal vascular response in healthy individuals through quantification of reactive hyperaemia which in turn can be a parameter to assess the decreased vascular response in Peripheral Vascular Disease.

Key Words: Vascular function, Pixel analysis software, Simple screening test

Introduction:

The cardiovascular system, along with the respiratory system, is the most important organ system for supplying nutrients to the tissues. All the metabolic activities, and in turn the "life" depends on this. Hence the integrity of both the Cardiac and Vascular component of this system needs to be maintained, as malfunction of any one or both will have disastrous effects. There have been many tests for assessment of cardiac performance, but the tests for vascular integrity and reactivity have not matched with that of cardiac function tests, in terms of accuracy, reliability, sensitivity and also ease of administration. The testing of vascular functions has gained importance as the incidence and prevalence of Peripheral Vascular Diseases (PVD) has been increasing (1).

PVD is commonly called peripheral arterial disease (PAD), which refers to the obstruction of large arteries not within the coronary or aortic arch vasculature. It is a manifestation of atherosclerosis characterized by atherosclerotic occlusive disease of the extremities and is a marker for atherothrombotic disease in other vascular beds.

Diabetes is one of the major causes of PAD, prevalence being more than three times higher in patients with Diabetes compared to non-diabetic persons and more so in chronic cases than newly identified ones at the baseline (2, 3). In people with diabetes, the risk of PAD is increased by age, duration of diabetes, and presence of peripheral neuropathy (4). Peripheral vascular disease affects 1 in 3 diabetics over the age of 50. Generally DM induced PAD affects the older age group but now-a-days there is an increased prevalence of the same in younger age groups as well because DM is reported to be increasing dramatically among young individuals. If this change in the epidemiology of DM continues, it is likely that a larger proportion of the youth population will have PAD in the future (5, 6).

If PAD is suspected, a number of tests need to be performed to detect the presence of atherosclerosis, as well as to localize areas of stenosis and to estimate the degree of the stenosis. Two types of techniques are used for the assessment of a patient with PAD: Noninvasive tests and Invasive tests. The main vascular laboratory tests are:

- Doppler velocity wave form analysis
- Angiography
- Color-assisted duplex ultrasonography
- Magnetic resonance angiography (MRA)
- Computed tomography angiography (CTA)

Each of these tests has its own advantages and disadvantages. In general the major disadvantages are high cost; difficult to carry out as they are technique intensive; non availability at the peripheral centers and even risks of adverse reaction and transmission of diseases (7).

Rationale for the present study

Data on the prevalence of diabetes induced PAD in the primary care setting are sparse, although this information is critically important as a scientific basis for developing strategies to enhance treatment of this condition and prevention of cerebrovascular and cardiovascular events in the community. Primary care is the principal target for investigation if the aim is improved population-based care. Primary care doctors play a key role, as they are the first point of contact for recognition, diagnosis and referral. Due to the availability of modern pharmacological and adjunctive therapy they are also increasingly important for the treatment of PAD.(8,9) However, there are several issues that urgently need to be addressed with new data in order to help design rational strategies to further improve the service provision and quality of care for PAD patients and of topmost priority in this regard is the large scale accessibility and availability of a non-complicated, noninvasive and less expensive method of diagnosis which can be easily and accurately handled even with minimal expertise.

The present study purports a simpler and non-invasive method of assessing the “change in the colour of the palmar-aspect of the hand with ischemia” and “reactive hyperaemia” as a diagnostic tool for the detection of presence of PAD in chronic diabetics.

Aim of the study

Qualitative and semi-quantitative assessment of the changes in the blood flow due to the phenomenon of the reactive hyperaemia by quantifying the colour changes as seen in the palm using a simple and non-invasive technique.

Objective

To observe and quantify the change in the colour of the palm with ischemia and reactive hyperaemia and compare with the other palm which is taken as control. This is done with the help of pixel analysis software “Chitradeepam” which is indigenously developed in the department of Physiology, K M C Manipal.

Methodology

Subjects

Healthy young students (5 males and 5 females) and Patients with Type 2 Diabetes Mellitus of more than 5 years’ duration, who were interested to volunteer in the study of “assessment of reactive hyperaemia through quantification of the colour changes” which was conducted in the department of physiology; Kasturba Medical College (KMC); Manipal; were selected as subjects. The study has been completed on healthy volunteers only.

Criteria for Selection of control subjects

- Healthy young adults in the age group of 20-25 years
- Subjects suffering from IDDM, HTN or any other ailment were excluded.
- Gender equality was maintained while choosing subjects.
- Subjects with fair complexion were preferred for sake of convenience.
- Subjects with any deformity and disfiguration in their palms were avoided.
- Too obese or too underweight subjects were excluded from the study.

Procedure

The experiment was done during the day time. The subject was sitting comfortably in a chair, completely relaxed in the air conditioned research laboratory and the full procedure was explained to the subjects. If willing he/she had filled in the consent form and only after that the procedure was started. After five minutes of rest, his/her blood-pressure was measured (using mercury sphygmomanometer) in the left arm and reported as systolic and diastolic-pressure. The mean of the three readings was used later for producing arterial occlusion. After five minutes of recording blood-pressure the subject was keeping the hand on the table on the area marked in such a way that both the palms would come in one frame of the camera (Sony digital camera, two mega pixel resolution was used) which was fixed on the camera stand. First four photos were taken at an interval of thirty seconds, which was used only as the base line data. After the base line recording, pressure in the left arm was increased 20 mm Hg above the systolic blood-pressure and maintained at a constant level. Photos were clicked at an interval of every thirty seconds until the subject complained of any discomfort. Whenever the subject complained of any kind of discomfort in the left hand, the pressure was immediately released completely. Photos were clicked of both the hands at an interval of around 5 seconds for a period of 2 minutes which was a total of 30 photos after the release of occlusion which corresponds with the period of the reactive hyperaemia. The cuff of the sphygmomanometer was removed and the subject was allowed to leave after five minutes. The images in JPEG format obtained were transferred to the laptop and converted to BITMAP format. Using the CHITRADEEPAM PIXEL ANYLYSIS SOFTWARE for each photo the number of red pixels in each photo was counted for the left hand and compared with the right hand values. The area selected for each hand for analysis was equal. Thus two sets of values were obtained i.e. one for the control hand and one for the experiment hand. The values were incorporated into the bar diagrams using MICROSOFT EXCEL and then documented. The results were analysed and accordingly interpreted also.

Statistical Analysis

The mean of the first four photographs for each hand represented the baseline value. The mean of all the photos during occlusion, which is of variable duration, represented the occlusion value. During post occlusion, the first minute value was separately calculated by analysing first 15 photos and the mean of the 15 photos represented the change at the end of first minute after release. Then the mean all the photographs of the post occlusion, in total 30 photographs including the first 15, represented the mean change of the post occlusion period.

The percentage change in the colour was analysed for first minute post occlusion and also at the end of 2 minutes' by calculating the change by comparing that respective value with the mean baseline value of that hand.

The mean values of the test hand of all the subjects (Males and Females) were compared with the corresponding values of the control hand using Student's 't' test for paired observations and $p<0.05$ was considered significant.

Results

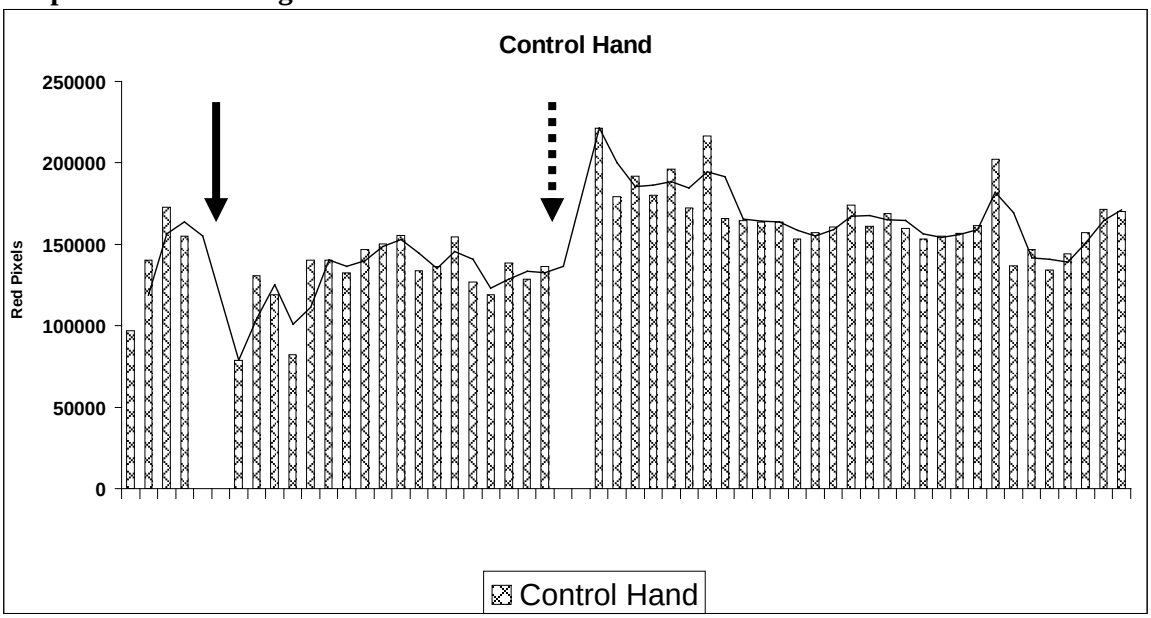
The study was conducted in 10 healthy subjects who were free from any kind of diseases and selected based on the inclusion and exclusion criteria already mentioned. Out of the 10 subjects selected, 5 were males and the other 5 were females.

Before the experiment was started, the height, weight and the baseline blood pressure (reported as systolic/diastolic) of each subject was recorded. Three readings were taken for the blood pressure and the mean of the three readings was used for producing the arterial occlusion.

For each subject, 3 sets of values were obtained, i.e. baseline data (4 values before occlusion), during ischemia (variable number of values) and post-ischemic period (30 values) for the control and test

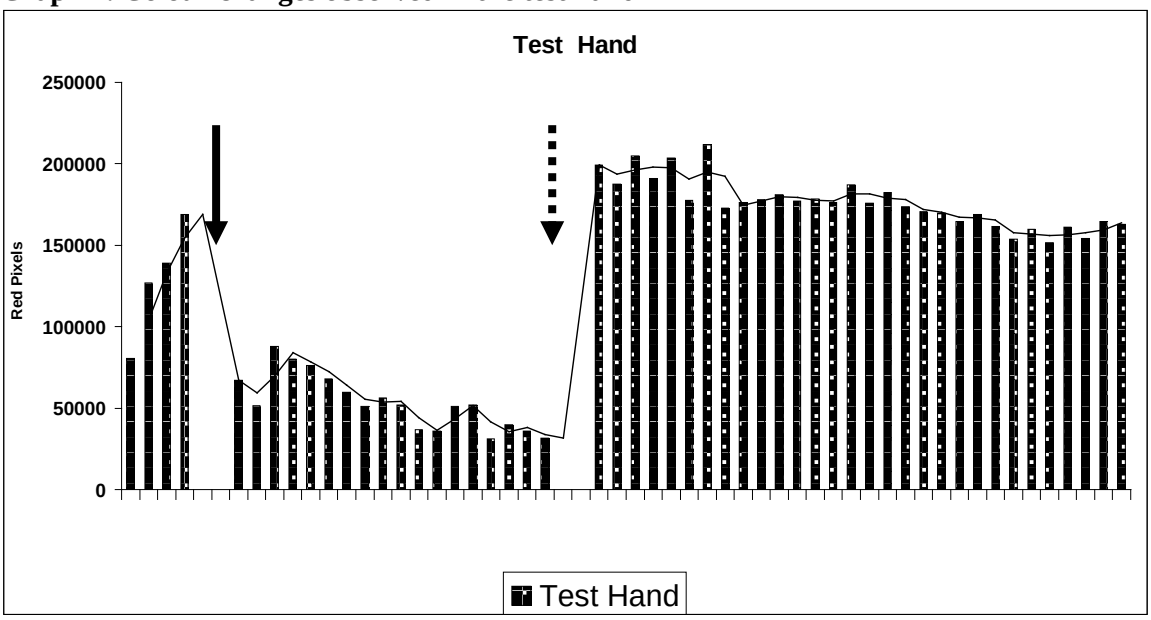
hand separately. A representative graph of an individual for the control hand, the test hand and for both hands together is given below.

Graph 1: Colour changes observed in the control hand



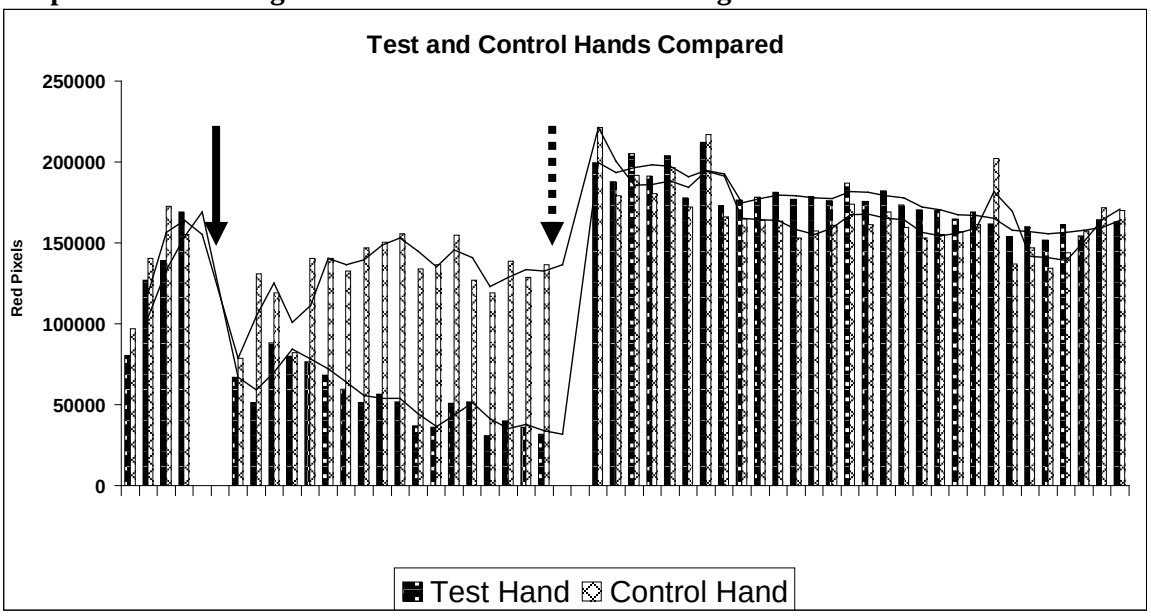
Solid Arrow– Occlusion started, Broken Arrow – Occlusion released

Graph 2: Colour changes observed in the test hand



Solid Arrow– Occlusion started, Broken Arrow – Occlusion released

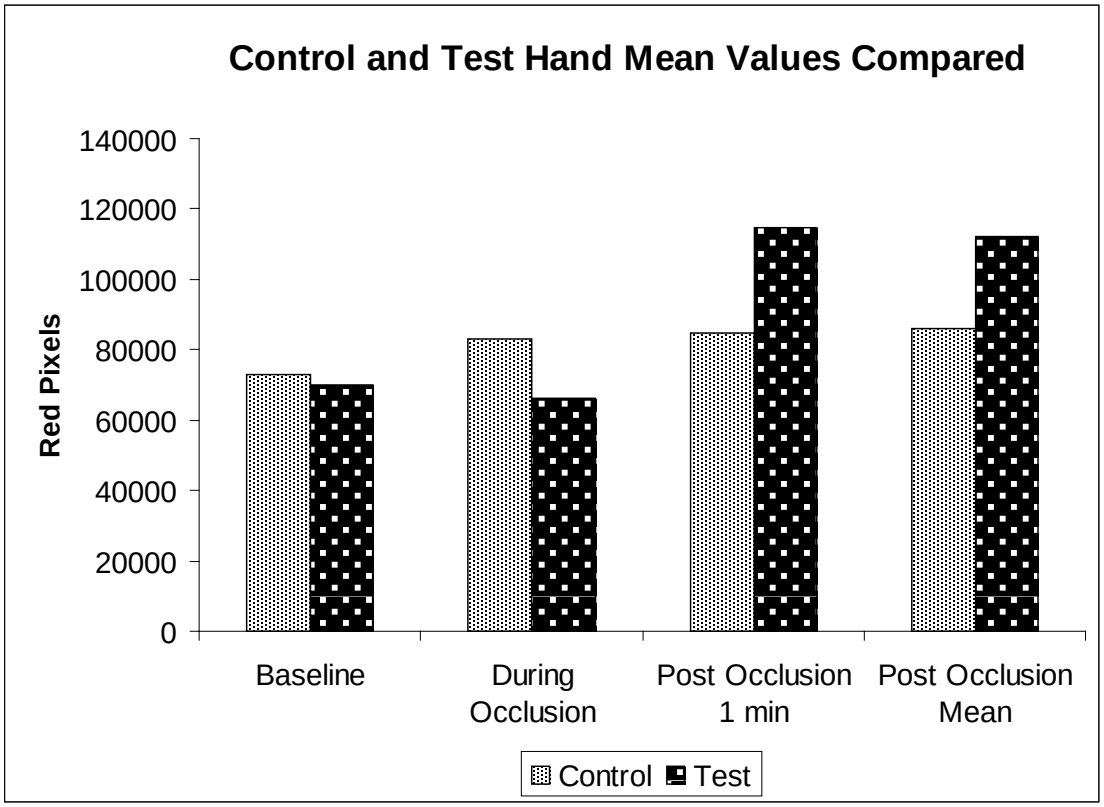
Graph 3: colour changes observed in both hands taken together



Solid Arrow– Occlusion started, Broken Arrow – Occlusion released

The mean was calculated for each of these stages separately. In the test hand, mean of the first four values (for all the subjects) served as the baseline data for that subject only. The same procedure was carried out during the period of ischemia, though this time period varied in each individual, depending up on the time for which they were able to tolerate it comfortably. During the post-ischemic period, mean was calculated for the first 1 minute (first 15 photos) and again an average of all the 30 photos in the post-ischemic period was calculated separately. Similarly the procedure was carried out in the control hand of all the subjects. A representative graph is shown below.

Graph 4: Colour changes Compared



All the mean values of each stage of control hand and test hand of all the 5 subjects in each group were tabulated. The mean and the standard deviations of those values were calculated and the paired t-test was used for the statistical-analysis. The values obtained were analyzed as shown below.
Table 1 and 2: Comparison of the values obtained in the control and the test-hand taken together and analyzed using Paired student's 't'- test.

Table 1: MALE SUBJECTS

No	Topics	Control hand (mean ± SD)	Test hand (mean ± SD)	p - value
1.	**Baseline**	**72778.3 ± 12602.07291**	**70105.5 ± 9010.29096**	**0.42168066**
2.	**During ischemia**	**83144.93953 ± 41439.19732**	**66203.54091 ± 20965.78187**	**0.37156512**
3.	**Post-ischemia (1min)**	**84710.45333 ± 25886.14198**	**114794.3867 ± 29444.83298**	**0.0195332***
4.	**Post-ischemia (mean)**	**85911.8423 ± 24542.78987**	**112351.9582± 25356.95444**	**0.0280362***
5.	**% Change Pre Vs Post1 min**	**21.5662395 ± 54.33920012**	**67.53944658 ± 58.14007126**	**0.0045972***
6.	**% Change Pre Vs Post Final**	**22.58375408 ± 49.88107477**	**63.55933163 ± 50.84734894**	**0.0067888***

(SD- standard deviation, *-p < 0.05 which is taken as significant).

Table 2: FEMALE SUBJECTS

No	Topics	Control hand (mean ± SD)	Test hand (mean ± SD)	p - value
1.	**Baseline**	**101725.9 ± 35693.69725**	**94673.4 ± 30530.50848**	**0.060679**
2.	**During ischemia**	**115187.6133± 38581.35804**	**68826.84 ± 31855.1013**	**0.0250490***
3.	**Post-ischemia (1min)**	**120308.4933± 47522.36298**	**132029.1333 ± 43562.83392**	**0.3852659**
4.	**Post-ischemia (mean)**	**117744.1044± 44672.04738**	**126036.9687 ± 45781.67196**	**0.4807511**
5.	**% Change Pre Vs Post1 min**	**19.87884078± 26.87505435**	**39.52435222 ± 15.18476165**	**0.30651334**
6.	**% Change Pre Vs Post Final**	**16.99207658± 21.18426156**	**31.25447227 18.89916251**	**0.4444394**

(SD- standard deviation, *-p < 0.05 which is taken as significant).

After analysis of all the 10 subjects, the following observations are made.

1. There are no significant differences in the colour of the baseline photos of the control hand and the test hand in both the groups.

2. During the period of ischemia, the two groups showed difference. In males, though there is a decrease in the colour of the test hand as denoted by the number of red pixels in the test hand, but the colour change is statistically not significant both when compared with the baseline data as well as with control hand. But in females, there was a significant difference between the test and the control hands during ischemia. This seems to be due to the combined effects of decrease colour in the test hand and a simultaneous increase in colour in the control hand, the increase denoting an increased blood flow during the occlusion of the test hand. **This is an interesting and novel observation**. The similar change was also seen in males.
3. In males during the first one minute of post-ischemia the test hand showed marked increase in the number of the red pixels, compared to the baseline and in the next 1 minute also, there was a significant increase in the number of the red pixels but the colour changes were less when compared to the first 1min. The average of the post-ischemic period values showed a significant increase in the number of red pixels (as indicated from the values) when compared with the baseline and also with corresponding values of the control hand. There was a similar trend in the females, though it was not to the same extent as seen in males and hence, was not statistically significant. This showed that there might be hormonal component to this effect, which needs to be further investigated.
4. The control hand also showed similar changes in the post ischemic period as that of test hand, but the magnitude was very less and statistically not significant compared to the baseline values.

Discussion and Conclusion

The transient increase in the blood flow that follows a brief period of arterial occlusion is called as the reactive hyperaemia. There are various methods which have been used to analyze the phenomenon of reactive hyperaemia. Some of these procedures are venous occlusion plethysmography, ultrasound-scanning and other methods. But most of these techniques are invasive. Moreover it requires well equipped tools and expertise to perform such techniques. Hence it is difficult to repeat these procedures over-time. Moreover the range of normal values also varied considerably, probably due to differences in the methodological-factors. Due to all these complications, the patient/subject compliance is also affected and it becomes difficult to use such techniques as a teaching tool or continue further researches in the long run.

In the present study, the change in the colour of the palmar aspect of the hand with ischemia was considered to assess reactive hyperaemia. For each subject 3 sets of values were obtained, i.e. baseline data (before occlusion), during ischemia (variable) and post-ischemic period (after the occlusion is released). As expected, there was a decrease in the number of red pixels during the period of ischemia compared to the baseline data, but the post ischemic period showed a significant increase in the number of red pixels, due to the phenomenon of reactive hyperaemia as assessed by the 'Chitradeepam' software used in the study as mentioned before. The colour change was more intense in the first 1 minute after the release of the occlusion. Though this colour change was still high, compared to the baseline in the next one minute as well, the magnitude of the change was less when compared to the initial 1 minute of the post ischemic period probably due to the fact that the blood supply was returning almost to the baseline levels. These finding are similar to the other results previously mentioned and show that the new method used in this study does compare with the other invasive methods previously used to study reactive hyperaemia.(10) Interestingly, in our study it was seen that the control hand also behaved in a similar manner, though the magnitude of the changes were much less when compared with test hand. The rapidity of the response indicates that there could be a neural component mediating this in addition to the metabolites released in the test hand, which are carried by the blood stream to bring about similar changes. This indicates that there is a scope to continue further research in this field to study the behaviour of the non occluded hand and since this method is non invasive and hence can be used extensively.

Our study also showed that there might be gender differences in the response. Male subjects showed a better vascular response to reactive hyperemia compared to females. This needs to be further looked into by studying more subjects and also the possible mechanisms.

Therefore the present study gives us a simple, non-invasive and a novel technique for the assessment of reactive hyperaemia. The test is easy to repeat overtime and the normal range of values

obtained also do not vary much as compared with the other complicated methods. So this method can be used as a teaching tool and also to continue further researches with a better patient/subject compliance in this field like analyzing reactive hyperaemia in patients with diabetes mellitus, peripheral vascular diseases like peripheral neuropathies and other disorders.

Conclusion:

In the light of the present study, it could be concluded that this is a very simple and non-invasive method with a possibility of similar sensitivity as the other procedures which are invasive and complicated being used for the assessment of reactive hyperaemia. Hence this technique should further be extensively tested in all age groups and both in healthy subjects & in patients with peripheral vascular diseases so that it can be used as a teaching tool and also a research tool.

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