End-to-end research evaluation needs to separate out the bibliometric part of the chain from the econometric part. Both size-dependent and size-independent terms play a crucial role to combine quantity and quality (impact) in a meaningful way. Output or outcome at the bibliometric level can be measured using zeroth, first or second-order composite indicators, and the productivity terms follow accordingly using the input to output or outcome factors.

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Fluorescence spectral features of blood components of pregnant women

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During pregnancy, women experience various metabolic and hormonal changes that contribute to foetal development. These changes are investigated in the present study in terms of fluorescent biomolecules found in blood using synchronous fluorescence spectroscopy. Comparing a set of blood samples of 14 pregnant women against age-adjusted controls, it could be seen that the amino acid tryptophan is approximately twofold higher in blood plasma of pregnant women ($P < 0.1$), while the metabolite flavin adenine dinucleotide is approximately 25% lower. Further, the essential oxygen-carrying protein in the haemoglobin, porphyrin, is 80% higher in pregnant women. When these results were compared with the spectral features of blood components of patients with thalassaemia, it was found that erythrocytes had approximately 25% less haemolysis during the tenure of pregnancy.

Keywords: Fluorescent biomolecules, pregnancy, red blood cells, synchronous fluorescence spectra.

SEVERAL dramatic changes in physiological and hematological conditions are known to occur during pregnancy[1]. Major changes in the blood include an increase in volume by 30–50% (ref. 2). This increase is progressive; it begins from the first trimester and peaks at around 32–36 weeks, with little change thereafter. This increase in blood volume is relatively greater than that in the red cell mass, resulting in the haemodilution state during pregnancy[2].

During pregnancy the red cell mass increases, which may be influenced by an increase in the maternal erythropoietin. The increase in red blood cell (RBC) production occurs to cope with the pregnancy demand and leads to a

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slight increase in mean corpuscular volume. The white blood cell (WBC) count is also increased, partly due to physiological stress induced by pregnancy. Neutrophils are the major WBC type that increase on differential count; yet phagocytic activity is decreased. There is also an increase in oxidative metabolism in neutrophils during pregnancy. Lymphocyte count decreases in the first and second trimesters, but increases during the third trimester. Generally, bone marrow shows an increase in erythropoiesis to cope with the needs during pregnancy[3]. Platelet count tends to decrease during pregnancy, especially during the third trimester, partly due to the haemodilution state as well as increased platelet activation and accelerated clearance[4]. Pregnancy is a hyper-coagulopathic state wherein significant changes occur in the hemostatic profile, including a progressive increase in fibrinogen and clotting factors. Although no significant changes occur in the bleeding time, partial thromboplastin time (PTT) is usually shortened in the third trimester[2].

Spectroscopy is a classic technique with a wide range of sub-disciplines. When photons interact with atoms or molecules, most of them are scattered (with or without changes in the wavelength) or absorbed. Some of the absorbed photons are converted into heat or re-emitted as fluorescence or phosphorescence. All the above spectral signals (Rayleigh and Raman scattering, UV–Vis absorption, fluorescence, phosphorescence) help us in understanding the biological processes of the human body. A variety of techniques such as infrared, UV–Vis absorption and Raman spectroscopy are in use for the diagnosis of a few diseases.

Fluorescence spectral diagnosis is a new technique to detect and diagnose diseases such as thalassaemia and sickle cell anaemia, by quantifying fluorescent biomolecules in blood plasma and erythrocytes[5–7]. Based on significant success, the technique is being used to explore the spectral features of blood components in healthy pregnant women in order to gain insights into the metabolic changes during pregnancy. In this study a comparison has been made in the spectral features of blood components of healthy normal control, healthy pregnant women and thalassaemia (Thal) patients.

The instrumentation and techniques employed in this study are similar to those previously described in the literature[8–11]. In order to highlight the major differences in spectral features of blood components in pregnant women, blood plasma and RBC samples were obtained from normal control and patients with thalassaemia.

A total of 37 subjects were considered for the study. Among these, 14 were pregnant women (8 in the first trimester and 6 in the second trimester) aged 20–35 years, 14 were non-pregnant women who were adjusted for age (normal control group), and 9 were patients with thalassaemia (17–34 years). All volunteers in the normal control group were regular employees of the King Saud University and the King Khalid University Hospital (KKUH), Riyadh, Saudi Arabia. They had no specific diseases, and the protocols of the study were explained to the subjects so as to obtain their informed consent. In addition, approval from the Scientific and Ethical Committee was obtained (E-15-1422) to collect samples from other subjects of KKUH.

Intravenous blood (5 ml) was drawn from each subject and collected in a vial coated with ethylenediaminetetraacetic acid (EDTA) on the inner wall. Each tube was gently inverted five times to mix evenly, followed by centrifugation (3000 rpm, 15 min) to separate the cellular components from the plasma. Approximately 1 ml supernatant plasma, a greenish-yellow liquid, was pipetted out and collected in a sterile glass tube. From the EDTA vial, the top buffy coat was carefully pipetted out and discarded; the thick, jelly-like residue contained cellular components, mainly erythrocytes. Next, 0.5 ml suspension of erythrocytes was lysed using 1.5 ml spectroscopic-grade acetone. The mixture was shaken well for efficient extraction of biomolecules from the erythrocytes. This was centrifuged (3000 rpm, 15 min) and the clear supernatant containing mostly fluorescent biomolecules was used for spectral analysis.

The spectrophotometer (Perkin Elemer LS 55, USA) used in the study is equipped with two diffraction gratings for emission, excitation or synchronous scans. The excitation grating can be preset to select light of a particular wavelength (e.g. 400 nm) and when used to excite a sample (e.g. acetone extract of erythrocytes), the emission grating is scanned from 425 to 700 nm to map the fluorescence profile of a set of biomolecules in that range. This is called fluorescence emission spectra (FES). On the other hand, when the excitation and emission gratings are set at 10 nm offset and rotated simultaneously, synchronous emission spectra (SES) of a host of molecules are obtained. These molecules include tryptophan, nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD) and porphyrin, which have partially overlapping emission profiles in complex systems such as the blood plasma. It is important to emphasize that FES and SES are variants of fluorescence spectroscopy[8,9], and that both spectra can be considered to be optical analogues of X-ray radiography and computed tomography (CT) scan[9]. On the other hand, when the offset between emission and excitation gratings is set at 70 nm, synchronous excitation spectra (SXS) of the above set of fluorescence molecules are obtained. SXS are also known as Stokes shift spectra[8]. It is important to emphasize that SES and SXS are complementary and hence the result of the first confirms that of the second.

Figure 1 shows the typical SXS of plasma for the three sets, i.e. normal control, pregnant women and Thal patients. Figure 1 *a* shows three major peaks at 290 (tryptophan), 370 (coenzyme NADH) and 450 nm (metabolite FAD). The intensity of the tryptophan peak was 40 and that of FAD was 8 arbitrary units. The ratio of fluores-

cence intensity for the peaks at 290 and 450 nm, $R_1 = I_{290}/I_{450}$ was 5 for the control group and 10 for pregnant women. This was because tryptophan was elevated twofold, though FAD was marginally enhanced during pregnancy. In contrast, for Thal group (Figure 1 *c*), tryptophan was found to decrease fourfold, while FAD was increased twofold. The corresponding ratio R_1 for the Thal group was 0.8.

Figure 2 shows the SES of blood plasma for the three sets. It is important to mention that SES and SXS are approximately mirror images: i.e. the 360 nm band in SES is the emission band for tryptophan and 530 nm is that of FAD (the corresponding excitation bands for tryptophan are at 290 and 450 nm, as shown in Figure 1). The results show that the intensity of the 360 nm band was approximately twofold higher for pregnant women compared to several fold lower for Thal patients. Similarly, the intensity of the 530 nm band was marginally lower for pregnant women and twofold higher for Thal patients, when compared to the normal control group.

The values of relative intensity ratio, $R_2 = I_{530}/I_{360}$ were 1.14 ± 0.21, 0.91 ± 0.08 and 8.00 ± 1.18 for the normal control group, pregnant women and Thal patients respectively. Additionally, $R_3 = I_{600}/I_{360}$ (the ratio of intensities at 600 nm due to porphyrin and 360 nm due to tryptophan) was found to be 0.23 ± 0.02, 0.15 ± 0.01 and 2.00 ± 0.20 for the normal control group, pregnant women and Thal patients respectively.

Figure 3 shows the FES of acetone extract of RBC for all three sets. Here, the band at 470 nm was due to the residual NADH, while those at 585 and 632 nm were due to two forms of haematoporphyrin. It is important to note that acetone strongly quenches the fluorescence of NADH. What we see is the residual fluorescence of NADH. Further, haematoporphyrin is a mixture of many derivatives with protoporphyrin IX as the principal fluorescing component. The 585 nm band is attributed to the basic and 632 nm to the neutral form of haematoporphyrin[10–12]. The intensity of the band at 632 nm was twofold higher for the pregnant women, but only 50% for Thal patients when compared to the normal control group. The values of relative intensity ratio, $R_4 = I_{632}/I_{585}$ were 1.10 ± 0.12, 2.00 ± 0.32 and 0.55 ± 0.05 for the normal control group, pregnant women and Thal patients respectively. In other

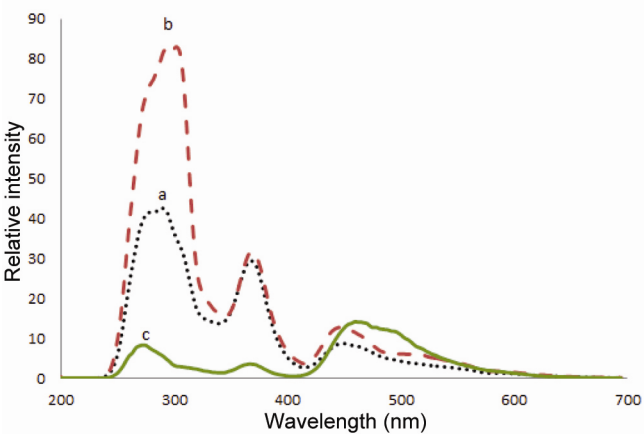

Figure 1. Synchronous excitation spectra of plasma ($\Delta\lambda = 70$ nm) in samples of (a) normal control; (b) pregnant women and (c) thalassaemia patients.

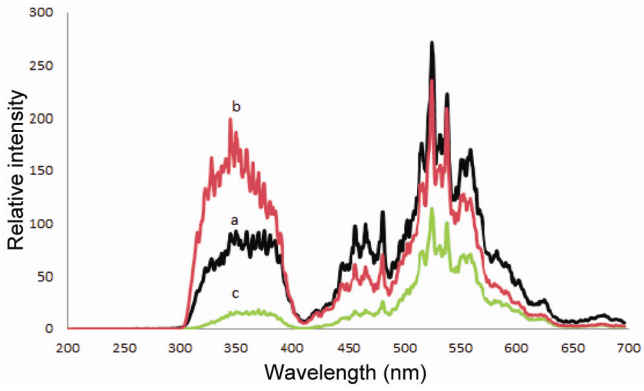

Figure 2. Synchronous emission spectra of plasma ($\Delta\lambda = 10$ nm) in samples of (a) normal control; (b) pregnant women and (c) thalassaemia patients.

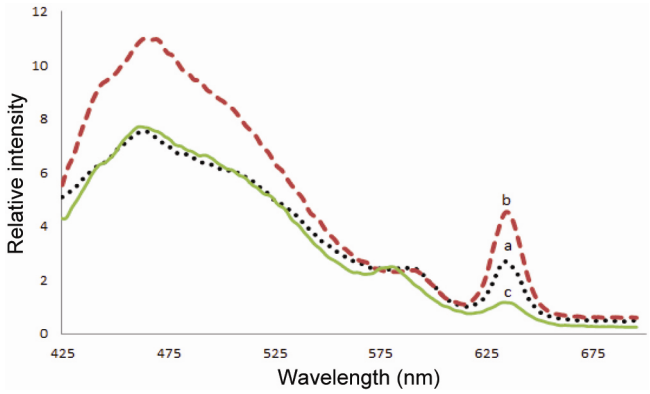

Figure 3. Fluorescence emission spectra of acetone extract of red blood cells (RBCs; excitation at 400 nm) in samples of (a) normal control; (b) pregnant women and (c) thalassaemia patients.

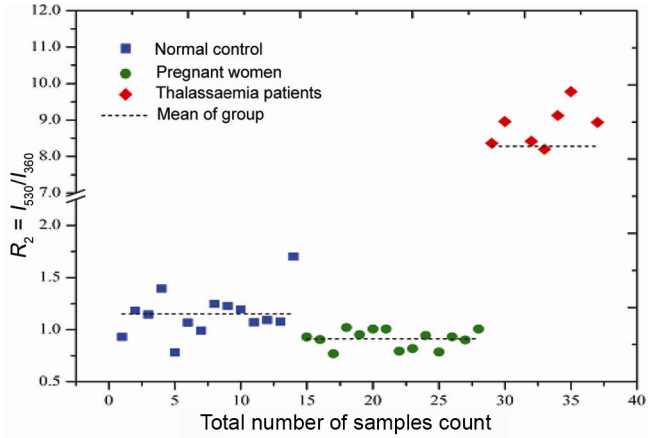

Figure 4. Distribution of $R_2 = I_{530}/I_{360}$. This ratio is a measure of FAD level in plasma as obtained from Figure 2.

words, porphyrin content in erythrocytes was 80% higher in pregnant women as compared to twofold lower in Thal patients.

Figures 4–6 show scatter plots for R_2, R_3 and R_4 respectively, for all the three sets, supporting the above results. Figure 4 shows that FAD content in pregnant women was only 75% compared to that in the normal control group, while it was 10-fold higher in Thal patients. Figure 5 shows that porphyrin in the plasma due to haemolysis was only 75% in pregnant women; however, it was eight-times higher in Thal patients. Figure 6 shows that porphyrin concentration, obtained after RBC lysis, was twofold higher in pregnant women and twofold lower in Thal patients.

Blood is a circulating fluid tissue that transports oxygen and nutrients to every cell in the human body and collects waste products for purification in the lungs and liver. Normal adult RBCs have a lifespan of 100–120 days[13,14], following which they are degraded to produce by-products such as biliverdin, bilirubin and porphyrins. The coenzyme NADH is involved in this degradation, with the consumption of NADH and production of the fluorescent component FAD (ref. 15).

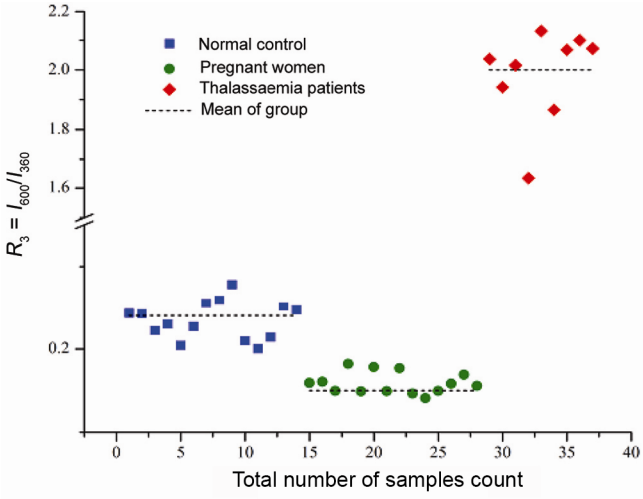

Figure 5. Distribution of $R_3 = I_{600}/I_{360}$. This ratio is a measure of porphyrin level in plasma as obtained from Figure 2.

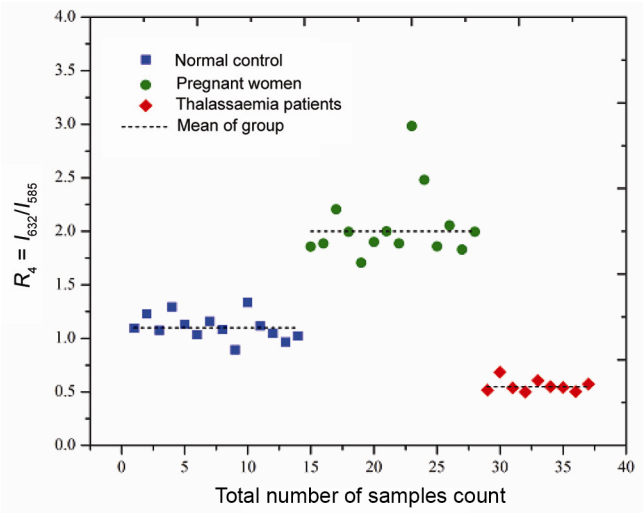

Figure 6. Distribution of $R_4 = I_{632}/I_{585}$. This ratio is a measure of porphyrin level in RBC as obtained from Figure 3.

Haemoglobin is the oxygen carrier to different cells of the body. It has two segments: heme and globin; the heme component contains protoporphyrin IX, which is made up of four pyrrole rings linked by methene bridges to form a tetrapyrrole ring. The iron atom lies in the centre of the protoporphyrin bound to the pyrrole nitrogen atoms. Under normal condition, the iron is in the ferrous (Fe^{2+}) state. It can form two more additional bonds, one on each side of heme plane. The iron lies approximately 0.4 Å outside the porphyrin plane because in this form it is slightly too large to fit into the porphyrin ring. This is deoxyhaemoglobin. When it comes in contact with oxygen in the lungs, there is a rearrangement of electronic cloud between oxygen and iron ions. This interaction is strong probably because both iron and oxygen are in the triplet paramagnetic state (both are tiny atomic magnets). Because of the changes in the electronic cloud, Fe ion gets gently sucked into the plane of the pyrrole ring. This in turn pulls the proximal histidine residue, bound to the fifth coordination site. Such changes are communicated to other parts of the large and complex haemoglobin, enabling the so called 'cooperative binding of oxygen'[16].

Haemoglobin becomes oxyglobin in the lungs with pH 7.4. However tissue sites of high metabolic activity (such as contracting muscle or foetus), generally have large amounts of hydrogen ions and CO_2, which leads to a local pH of 7.2. When oxygen is released from the lungs, it has a pH of 7.4 and partial pressure of 100 torr; but when it reaches the tissue target, with pH of 7.2 and partial pressure of 20 torr, 77% of oxygen is transferred to every cell of the tissue sites of the foetus[16]. This description is provided to show how porphyrin content in the RBC is directly proportional to the oxygen-carrying capacity.

In order to understand the spectral features of blood components in pregnant women, we need to consider two distinct features of thalassaemia, an inherited disease, totally unconnected to pregnancy. The first is that thalassaemia is well known for reduced RBC count (Table 1). This has been well-substantiated by the results obtained from independent spectral analysis performed in this study (Figure 3), where R_4 value for Thal patients was half that obtained for the control group[16]. The second important factor is that the RBC pool in a Thal patient is composed of immature reticulocytes and microcytes, which are small in size. This contributes to faster degradation and a shorter lifespan of 60 days[17,18]. This fragility of RBCs is demonstrated in Figures 1 and 2, where tryptophan (an essential amino acid) level was substantially low and metabolite FAD was significantly high, leading to a sixfold lower value of R_1 in Thal patients.

Table 1. Red blood cell analysis by complete blood count in normal control, pregnant women and thalassaemia patients.

	Red blood cell in (10^{12}/l)		
	Normal Control	Pregnant women	Thalassaemia patients
	4.78	5.12	2.49
	5.2	4.9	2.98
	5.26	4.3	2.82
	4.69	4.42	2.87
	5.31	4.43	2.84
	4.25	4.49	2.93
	4.56	4.38	2.79
	4.89	4.49	2.84
	5.2	6.27	3.05
	5.12	4.24	
	4.48	4.29	
	5.39	4.64	
	4.82	4.74	
	5.26	4.25	
Average	5.007857143	4.64	2.845555556
SD	0.362728518	0.534947877	0.157250685

From the study of spectral features of Thal patients, who exhibit 50% higher level of haemolysis[5–7], we could infer that pregnant women manifest 25% lower level of haemolysis. This was demonstrated by three corroborating observations: first, tryptophan level was approximately twofold higher; secondly FAD level was approximately 25% lower in the pregnant women compared to the control group (Figure 1) and thirdly, porphyrin level was 80% higher in pregnant women (Figure 4). Since there is a huge demand for oxygen required for the growth of the foetus, the inbuilt cellular control mechanism could send a signal for additional production of RBCs. However, as shown in Table 1, the RBC level of most pregnant women was about 10% less than that of normal control group; leading to the hypothesis that the enzymes and hormones during pregnancy provide extra fortification for porphyrin. The present study also indicates that the hemolytic products are significantly less for pregnant women. In short, pregnancy is the opposite of thalassaemia, as far as haemolysis and oxygen-carrying capacity of RBC are concerned.

It is important to emphasize that the above results need to be confirmed by further studies in the future.

This study compared the spectral features of fluorescent biomolecules dispersed in different components of the blood in pregnant and non-pregnant women. In order to gain proper understanding, the results were compared with those of thalassaemia patients. The results of this study indicate that RBCs show higher porphyrin content and less haemolysis for pregnant women compared to normal control group. That is, blood features in pregnant women are the opposite of thalassaemia patients. Further studies are under way to confirm the above results.

Competing interests: The authors declare that they have no competing interests.

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