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Contact Details:
Professor Ihsan M. Ajeena
Editor in Chief
e-mail: kufamed.journal@uokufa.edu.iq
Secretariat Phone Number: +9647801763952
Homeland Mail Address: Faculty of Medicine, University of Kufa, PO Box 21, Kufa, Najaf, Iraq

Editorial Team

Editorial

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Professionalism

by Shabih H. Zaidi, NHS- UK, SIVU (www.SIVU.alsadiquni.com)

Professionalism is a multifaceted attribute that affords a distinct place to a person in a society on the basis of specialized knowledge and matching skills ; and is the third dimension of rights and obligations. It expects competence in one's profession, problem solving capability, and resolving issues of the people, cohorts, peers, juniors or employees. It is also manifested in the shape of respect earned while giving it to others.

A professional earns respect through hard work, dedication, commitment and devotion duly checked and balanced with accountability. That is why the medical profession has a system of appraisal and audit, which follows the guidelines set up by the registering national authority. Self-regulation is an important component of professionalism. The professional oaths are given to physicians at graduation, which duly highlight such elements as care of the sick with proficiency, special care of the minors, disabled and otherwise incompetent, respecting patients and human dignity, avoiding abuse of expertise, avoiding personal malice, grudge, stigmas, discrimination and greed.

Professionalism enjoys a certain status in every society. Formulation of professionalism from an Islamic perspective consists of seven dimensions of faith (*iman*), consciousness (*taqwa*), best character (*ahsan al akhlaq),* excellent performance (*itqaan al 'amal*), strife toward perfection (*ihsan*), responsibility (*amanat*), and self-accountability (*muhasabat al nafs*)[(1)].

In his famous epistle recorded in Nahjul Balagha, the philosopher- soldier of Islam , Imam Ali gave a series of commands on good governance and professionalism to his Governor Malik-e-Ashtar. (2)

Salient components of professionalism are:

1.*Physician-Patient Relationship* is the fundamental pillar of medical professionalism. Several models are identified by the savants which can be tailored to meet cultural needs[(3-4)]:1) The *Paternalistic Model* in which the doctor knows it all, 2)The *Informative Model* is where information is shared, 3) The *Interpretive Model* in which the physician provides all the relevant information including the need for or results of diagnostic tests, 4) The *Deliberative Model* is where deliberations are to be focused during consultation, more as a friend than a professional pundit sitting on a high altar.

2.*Ethics* is the fundamental brick of professionalism. *Trust* is its very soul. It plays a pivotal role in patient - physician relationship. This relationship was paternalistic for a long time as a patient believed that a physician will do his best to relieve him of his ailments (Beneficence) without causing any harm (Non-maleficence).

Autonomy is a pillar of ethics which encompasses the right of persons to freedom of conscience and to respect as agents capable of making their own judgments in accord with universal moral principles, or in accord with freely arrived at decisions.

3.*Informed Consent* is the backbone of autonomy, and the process in which a health care provider educates a patient about the risks, benefits, and alternatives of a given procedure or intervention. It has many shades [(5)].

4.*Confidentiality* is an extremely important component of medical professionalism that means not sharing the personal information gathered during the consultation between the healthcare worker and the person or indeed with other professionals (6)and a breach of privacy is the outcome of a defective moral compass.

5.*Conflict of Interest* can sometimes tarnish professionalism. A common example could be seen when a physician employed by a health authority brings disrepute to the parent organization through his words or action. Referring a patient from the public sector to his private practice is a common example.

6.*The COVID-19* demolished the world; however, a silver lining is the explosion of digital technology, asynchronous education and health care through Telemedicine. Telehealth on the other hand covers administrative and managerial matters, CME, educational activities and training of the professionals in improving the health care, and medical professionals should harness it.

7.*Managing medical errors* is part and parcel of medical professionalism. A physician is as liable to make mistakes as any other human being. It is nearly always unintentional and unintended. If it happens, there are strict guidelines in every country promulgated by the licensing authority to document and deal with it accordingly. Defensive medicine is the outcome of lack of indemnity in many countries.

8.*The Patient-Physician financial relationship* is another element to consider. Medical profession, just like any other profession, is justified in charging a fee for services. What is however important is that the charges must be commensurate with the level of service as well as affordability.

In conclusion, professionalism is a multifaceted quality .The main characters of which include a pleasant demeanor, an ethical approach , being truthful and loyal, competent and expert, with strong communication skills, an exemplary behavior, a poised and balanced personality, being honest, fair, just and a life-long learner.

Professionalism is inseparable from the medical profession and must be incorporated in an undergraduate and graduate curriculum.

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Serum Mannose Level and Its Association in Women with Polycystic Ovarian Syndrome: a Case Control Study of Al-Najaf City / 2020

Basima Shamkhi Al-Ghazali [(1)], Sara Monther Al-Kuaity [(2)]

[(1)]Obstetrics & Gynecology department, College of Medicine, Kufa University, [(2)]Al-Zahraa Teaching Hospital

Corresponding Author: Basima Shamkhi Al Ghazali: basima.alghazali@uokufa.edu.iq

Abstract

Background: Polycystic ovarian syndrome is a common public health issue around the world. Insulin resistance and glucose intolerance play important role in the etiology of polycystic ovarian syndrome and the serum mannose is incorporated in metabolism of glucose.

Aim of study: Determination of the association between serum mannose level and polycystic ovarian syndrome.

Patients and Methods: A case control study enrolled eighty eight women were divided as forty four women with polycystic ovarian syndrome and forty four women without polycystic ovarian syndrome presented to outpatient clinic at Fertility Center/Al Sader Medical City and outpatient clinic of Al- Zahraa Teaching Hospital in Najaf city-Iraq during the period from 1st of February till 31st of December, 2020.

Results: The mean Serum Mannose level of women with polycystic ovarian syndrome 7.37 was significantly higher than mean serum mannose of controls 1.32 ($p<0.001$). Cutoff serum mannose level of 1.98 had acceptable validity results (90.9% sensitivity, 90.9% specificity, 94% PPV, 90.5% NPV and 90% accuracy). The means of serum level testosterone and luteinizing hormones were significantly higher among women with polycystic ovarian syndrome, while mean follicular stimulating hormone level was significantly lower among women with polycystic ovarian syndrome.

Conclusions: The serum mannose level is significantly increased in women with polycystic ovarian syndrome which may indicate its possible role in the pathophysiology of polycystic ovarian syndrome.

Keywords: Polycystic ovarian syndrome (PCO), testosterone, luteinizing hormones, follicular stimulating hormone, Serum Mannose.

Introduction

Polycystic ovarian syndrome (PCOS) is the most common endocrine pathology and is a leading cause of ovulatory dysfunction in the reproductive-aged female around the world were its prevalence ranges around 5% to 15% depending on the diagnostic criteria. It is a hyperandrogenic state with oligoanovulation that cannot be explained by any another disorder and is diagnosed by exclusion. [1]. The main pathophysiology of PCOS is still unknown but several theories have been proposed to explain the pathogenesis of PCOS [2] and ovarian hyperthecosis and hyperandrogenism in addition to insulin resistance are considered the main pathological disturbance in PCOS, in addition to numerous genetic and environmental factors which has been postulated to interact and play a role in the underlying pathophysiology of this syndrome. Limited data are available regarding newer biomarkers, except for Anti-Müllerian hormone (AMH) and several other biomarkers like testosterone which may be associated with PCOS.

Mannose is predominantly monosaccharide for protein glycosylation and is account for the majority of mannose found in mammalian blood [5,6]. The importance of mannose in PCOS-related metabolic disorders has been increasingly recognizeed, for example, plasma mannose levels are significantly elevated in women with insulin resistance [6,7]. In addition to that obese women with metabolic abnormalityes express a significant decline in mannose metabolism and utilization genes in the livers [6,7].other studies found that elevated plasma mannose levels may be associated with common chronic diseases like type 2 diabetes and cardiovascular disease, which may be used as a predictive biomarker [8]. Despite these advances in knowledge, the role of mannose in the pathogenesis of PCOS remains not clear [8]. So the aim of the present study is to determine the association between serum mannose level and polycystic ovarian syndrome.

Patients & Methods

A case control study was done at Fertility center/ Al- Sader Medical City and Al-Zahra'a Teaching Hospital for Maternity and Pediatrics /Najaf/Iraq, during the period from 1st of February till 31st of December, 2020.The protocol of the study was approved by scientific council and ethical committee of the Iraqi board of medical specialization were 88 women included in the study after an informed consent. The study sample consisted of 44 PCOS women and 44 controls their age between 18-45 years. The final diagnosis of polycystic ovarian syndrome was done according to Rotterdam criteria [9]. The exclusion criteria are tobacco smoking, hormonal treatment such as oral contraceptive pills, gonadotropins releasing hormones agonist or antagonist, hyperprolactinemia, endocrine disorders such as Cushing's syndrome, congenital adrenal hyperplasia, hypothyroidism pituitary tumors and refuse to participate.

After explanation of the whole procedure, patients' information was documented in details in a prepared questionnaire including age, parity, family history drugs history, surgical history, smoking habit, menstrual history. A physical examination and baseline assessment was done for the patients at the day 2 or 3 of menstrual cycle; BMI was calculated as body weight in kilograms divided by height in squared meters (kg/m^2) [10]. Transvaginal ultrasound scan was performed for all participants. A blood samples were withdrawn at the first 2-3 days of the menstrual cycle and

centrifuged to collect serum. Part of the serum was used to measure hormones including FSH, LH, serum prolactin and total testosterone.

Total serum mannose was assessed in an enzyme linked immunosorbent assay (ELISA). All women's data analyzed using computerized statistical software; Statistical Package for Social Sciences (SPSS) version 22 was used. Descriptive statistics presented as (mean ± standard deviation) and frequencies as percentages. Multiple contingency tables conducted and appropriate statistical tests performed, Chi square test was used for categorical variables (Fishers exact test was used when expected variable was less than 20% of total variable). Independent sample t-test was used to compare between two means. ROC curve was used for prediction of PCOS by serum mannose and testosterone levels. In all statistical analysis, level of significance (p value) set at ≤ 0.05 and the result presented as tables and/or graphs.

Results

This study included forty-four women with polycystic ovarian syndrome (PCOS) with mean age of 27.8±4.9 years; mean age of women with PCOD was insignificantly higher than mean age of control women (p=0.14). No significant differences were observed between women with PCOS and controls regarding marital status (p=1.0) and parity (p=0.8). There was non-significant difference in body mass index between women with PCOS and controls (p=0.16); 27.3% women with PCOS were obese, while 13.6% of controls were obese. Mean BMI of women with PCOS was non significantly higher than mean BMI of control women (p=0.25) (Table 1).

Mean serum testosterone level of women with PCOS (0.74 ng/dl) was significantly higher than mean serum testosterone of (0.24 ng/dl) for controls (p<0.001). Mean follicular stimulating hormone level of women with PCOS was significantly lower than mean follicular stimulating hormone of controls (p=0.001). Mean luteinizing hormone level of women with PCOS was significantly higher than mean luteinizing hormone of controls (p<0.001). Mean serum mannose level of women with PCOS (7.37±4.68 ng/ml) was significantly higher than mean serum mannose of (1.32±0.74 ng/ml) for controls (p<0.001). (Table 2) (Fig.1, 2, 3). The cut off serum mannose level of 1.98 had acceptable validity results (90.9% sensitivity, 90.9% specificity, 94%PPV, 90.5% NPV and accuracy 90%) while the cutoff serum testosterone level of 0.55 had acceptable validity results (93.2% sensitivity, 100% specificity, 92.5%PPV, 100% NPV and accuracy 95%) and the cutoff serum LH level of 8 had acceptable validity results (80% sensitivity, 91% specificity, 89.7%PPV, 81.6% NPV and accuracy 85.2%) (Table 3) (Fig. 4,5,6).

Discussion

Hyperandrogenism is accompanied with a profound risk of insulin resistance, metabolic syndrome and liver steatosis which are all features of polycystic ovarian syndrome (PCOS) [11,12]. Some authors detected a link between testosterone and glucose intolerance, among women with PCOS [13]. The serum mannose is important peptide that is associated significantly with hyperandrogenism and insulin resistance [14].

The present study found that mean serum mannose level of women with PCOS was significantly higher than mean serum mannose of controls (p<0.001). This finding is similar to results of Mi *et al* [15] study in USA which stated that serum mannose level is directly linked to androgens especially higher levels are detected in polycystic ovarian syndrome.

Table 1: Demographic characteristics of the study groups.

Variable	Study groups				P value
	PCO		Control		
	No.	%	No.	%	
Age					0.2*
<20 years	0	-	1	2.3	
20-29 years	27	61.4	32	72.7	
30-39 years	17	38.6	11	25.0	
Mean±SD (years)	27.8±4.9		26.3±4.5		0.14 **
Body mass index					0.16***
Normal	8	18.2	14	31.8	
Overweight	24	54.5	24	54.5	
Obese	12	27.3	6	13.6	
Mean±SD (Kg/m^2)	27.7±3.00		26.9±3.4		0.25**
Marital status					1.0*
Married	34	77.3	34	77.3	
Single	10	22.7	10	22.7	
Parity					0.8***
Nulliparity	10	22.7	10	22.7	
Primi-parity	8	18.2	8	18.2	
Multi-parity	18	40.9	21	47.7	
Grand-multiparity	8	18.2	5	11.4	
Mean±SD	2.2±1.8		2±1.7		0.6^{**NS}

*Fishers exact test,**Independent sample t-test, ***Chi-square test, NS=Not significant. P value <0.05 was significant

Table 2: Distribution of serum luteinizing hormone, follicular stimulating hormone, testosterone and mannose level ac- cording to study groups.

Variable	Study groups		P value
	PCO	Control	
	Mean± SD	Mean±SD	
LH (IU/L)	9.41±3.64	5.75±1.52	<0.001
FSH (IU/L)	6.65±2.62	8.79±3.24	0.001
Serum testosterone(ng/dl)	0.74±0.1	0.24±0.09	<0.001
Serum mannose (ng/ml)	7.37±4.68	1.32±0.74	<0.001

*Independent sample t-test, P value <0.05 was significant

Table 3: ROC coordinates for prediction of PCO by serum man- nose, testosterone and LH level

ROC coordinates for prediction of PCO by serum mannose level					
Cutoff point	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
1.8	93.2%	77.3%	90.8%	80.5%	86%
1.98	90.9%	90.9%	94%	90.5%	90%
2.2	81.8%	97.7%	88.8%	93%	85%
ROC coordinates for prediction of PCO by serum testosterone level					
Cutoff point	Sensitivity	Specificity	PPV	NPV	Accuracy
0.46	100%	55%	100%	57%	73%
0.55	93.2%	100%	92.5%	100%	95%
0.61	84.1%	100%	80%	100%	89%
ROC coordinates for prediction of PCO by serum LH level					
Cutoff point	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
7	77%	60%	74%	72.2%	68.1%
8	80%	91%	89.7%	81.6%	85.2%
9	61%	90%	87.1%	70.1%	76.2%

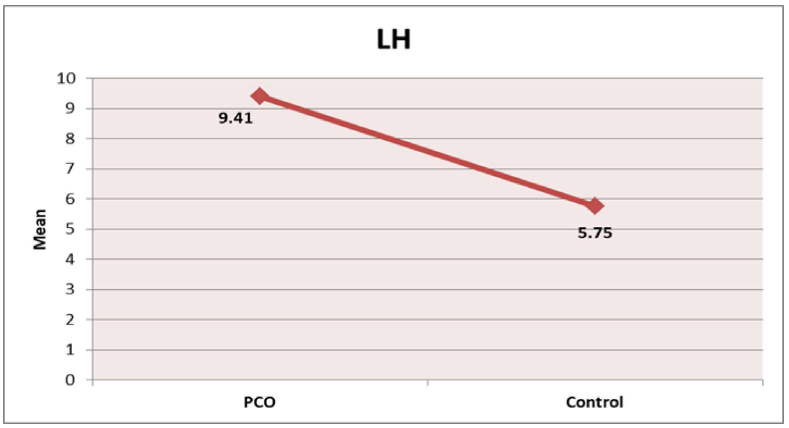

Figure 1. Distribution of serum LH mean according to study groups.

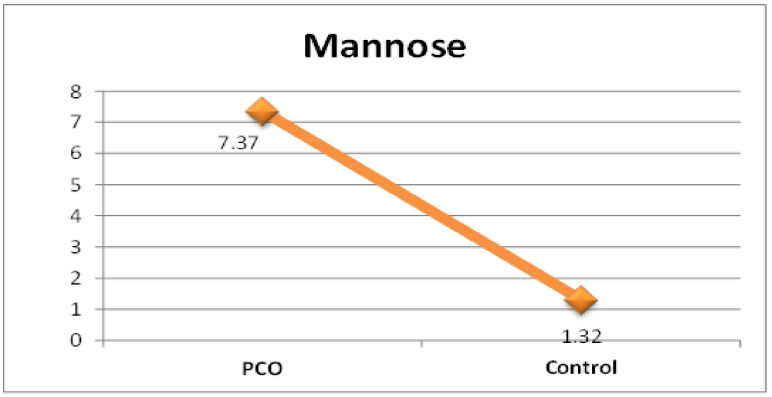

Figure 2. Distribution of serum mannose mean according to study groups.

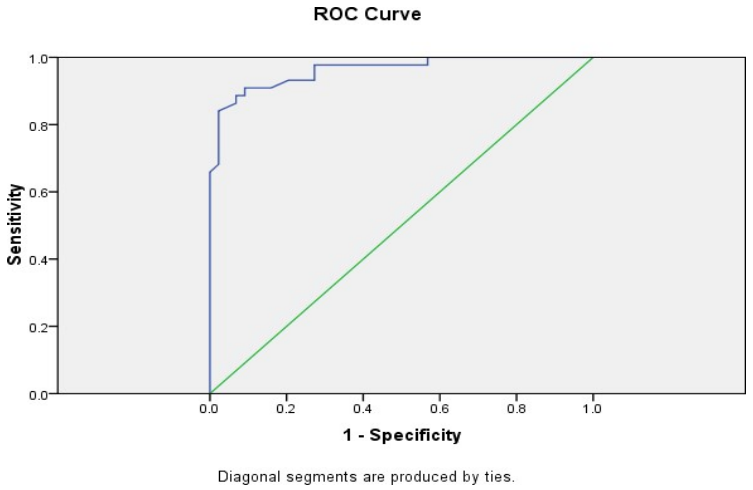

Figure 3. ROC for serum mannose level prediction of PCO (AUC=0.96).

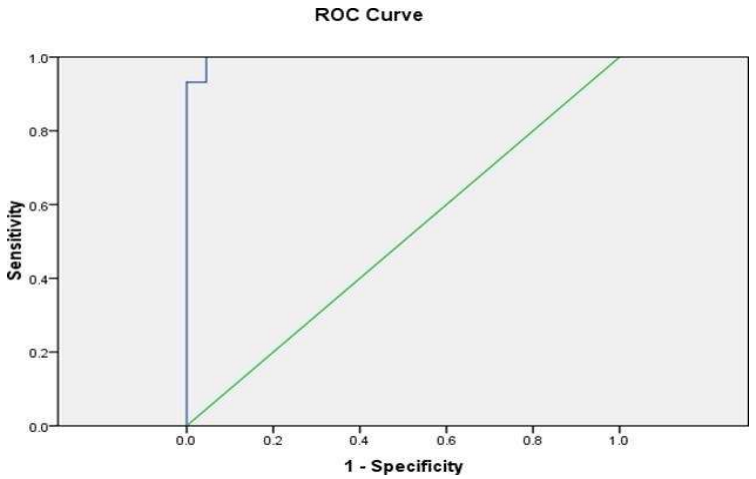

Figure 4. ROC for serum testosterone level prediction of PCO (AUC=0.99).

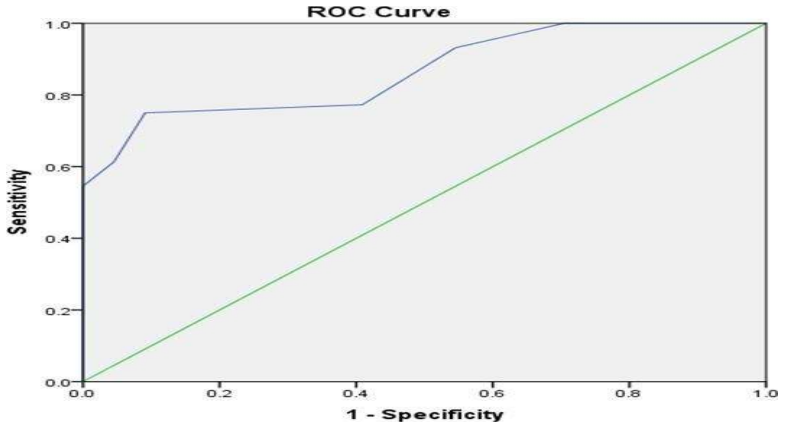

Figure 5. ROC for serum LH level prediction of PCO (AUC=0.865).

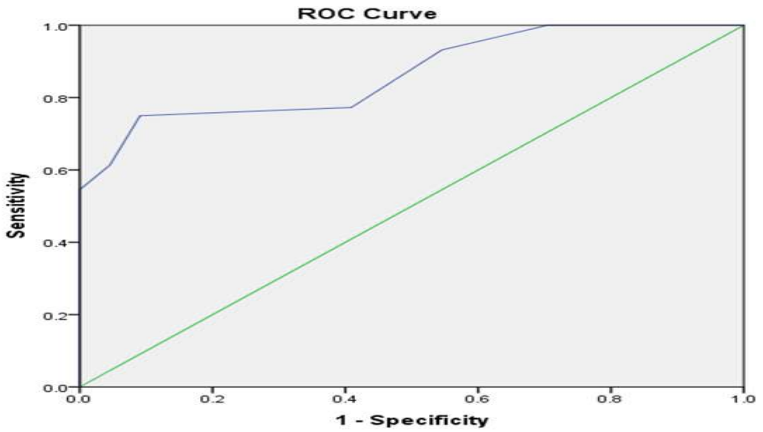

Figure 6. ROC for serum LH level prediction of PCO (AUC=0.865).

However many authors reported a novel relationship between serum mannose level with both of testosterone and luteinizing hormones in PCOS women. Witchel *et al.,* [16] study in USA document-ted that glucose abnormality like high serum mannose level and insulin resistance play an important role in etiology of polycystic ovarian syndrome. Another American study carried out by Hsiao *et al*., found a significant relationship between excess of androgens and higher level of serum mannose especially among women with polycystic ovarian syndrome. Kowalska *et al.,* [17] study in Poland documented that serum mannan binding lectin is markedly declined among women with PCOS and no signific-ant relationship was observed between serum mannose level and androgens.

Current study found that serum mannose level of 1.98 ng /ml had accept-able validity results (90.9% sensitivity, 90.9% specificity, 94% PPV, 90.5% NPV and 90% accuracy). Feng et al [18] on 71 women with PCOS and 61 healthy women which revealed that serum mannose was increased among women with PCOS and serum mannose could predict the polycystic ovarian syndrome with accept-able validity findings (66.2% sensitivity, 73.8% specificity, 74.6% PPV, 65.2% NPV and 68% accuracy). Peña et al [19] study in Cuba found that insulin resistance is significantly increased among women with PCOS than women with normal ovarian function. A Japanese study by Yoshimura *et al.,* [20] showed a profound relationship between serum mannose level and glucose tolerance in humans. For that, the serum mannose level could be used as a significant predictor for early diagnosis of polycystic ovarian syndrome [20].

In this study, mean serum testosterone level of women with PCOS was significantly higher than mean serum testosterone of controls ($p<0.001$). This finding coincides with results of Alsaadi and Mohamad study [21] in Iraq which found that mean testosterone level was significantly higher among women with PCOS as compared to healthy women, It is also consistent with results of Lerchbaum et al [22] in Austria which documented those PCOS women is significantly related with high serum testosterone level and the higher levels of free testosterone in PCOS women is indicator of adverse metabolic reactions. Zhang *et al.,*[23] study in China found that androgen hormones increase (such as testosterone) is directly linked to increase in glucose intolerance as these

hormones increased the insulin resistance and beta cell dysfunction among women with polycystic ovarian syndrome. Our study found that serum testosterone level of 0.55 had acceptable validity results (93.2% sensitivity, 100% specificity, 92.5% PPV, 100% NPV and accuracy 95%), which is in agreement with results of Iwasa et al [24] study in Japan which revealed an important role in serum testosterone level of 0.71 for diagnosis of polycystic ovarian syndrome with acceptable validity results (78% sensitivity and 54% specificity) [24].

Regarding mean luteinizing hormone level of women with PCOS we detected that it is significantly higher than mean luteinizing hormone of controls ($p<0.001$) and the serum LH level of 8 iu/l had acceptable validity results (80% sensitivity, 91% specificity, 89.7%PPV, 81.6% NPV and accuracy 85.2%). Kanamarlapudi *et al.,* [24] study in UK reported that luteinizing hormones were over-expressed among women with polycystic ovarian syndrome and Nicholas *et al.,*[25] study in USA clarified the relationship between luteinizing hormone and insulin resistance.

Conclusion

The serum mannose level is significantly elevated in polycystic ovarian syndrome which might indicate its role in pathophysiology of polycystic ovarian syndrome.

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Competing interests

The authors declare that there is no conflict of interest.

Author Contributions

The authors prepared the questionnaire, collect and analyses the cases, wrote, read and approved the final manuscript.

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Evaluating the Anti-Cancer Effect of Pomegranate Aqueous Extract of Gold Nanoparticles by Using Photodynamic Laser on Laboratory Mice.

Entidhar Jasim Khamees

Department of Physiology and medical physics, College of Medicine, University of Babylon

Email: intdher071@gmail.com, med.intidhar.jasim@uobabylon.edu.iq

https://orcid.org/0000-0001-6149-5455

Abstract

Background: This study evaluated the anticancer effects of gold nanoparticles (Au-NPs) generated by reducing hydrated gold metal ions in the presence of aqueous pomegranate peel extract.

Aim of study: To test the possibility that Au-NPs generated from the pomegranate aqueous extract could be employed as an anti-cancer therapy.

Materials and methods: The study aimed to evaluate Photodynamic therapy (PDT) using a laser at a wavelength of 532 nm with green gold particles as a photosensitizer in in-vivo experiment. Gold nanoparticles (Au-NPs) were produced by reducing hydrated gold metal ions in the presence of aqueous pomegranate peel extract. Using UV-visible spectroscopy, the absorption peak of the gold particles was found near 550-592 nm. All diffraction peaks for 2θ at = 37.8227º at 43.7014º, 63.8103º and 77.1540º correspond to the (111), (200), (220), and (311) levels, respectively by X-ray diffraction (XRD) analysis, imaging confirmed Au-NP by Transmission electron microscopy (TEM), the nanoparticle size ranged between 19.96 ± 0.784 nm. Forty Mature Swiss females weighing 20–25 g and aged 6–8 weeks were used, the animals were placed in well-ventilated rooms.

Results: The best exposure time to eradicate the tumor is with a duration of exposure of 30 minutes using the diode laser, as histological investigations indicated the development of anti-immunity with the same exposure period.

Keywords: Gold Nanoparticles, Pomegranate Aqueous Extract, Histopathological, Tumorbearing female mice.

Introduction

Nanotechnology is a field of science concerned with the investigation of materials in the nanoscale, which ranges from 1 to 100 nanometers. It is a nanoscale science that provides numerous focus points to many disciplines of research such as dentistry, pharmacology, and bioengineering [1]. Scientific research on the manufacture of metal nanoparticles from natural sources such as plants [2], fungus [3], algae [4], bacteria [5], and viruses [6] has expanded during this time.,Because of their oxidation resistance, stability, and biocompatibility, Gold nanoparticles (Au-NPs) are among the most significant nanomaterials. Food packaging [7,8], biomedical [9], medication delivery, diagnostics, imaging [10], and cosmetics can all benefit from Au-NPs [11]. Several techniques have been tried for the green synthesis of gold NPs. Various quantities of Aloe vera (L) Burm. f. leaf extract were added to 6 mL of chloroauric acid (HAuCl4) and distilled water was added to make the compound up to 10 mL. For Au-NP synthesis, the reaction mixture was permitted to stand for 30 hours [12]. A Musa acuminata Colla peel extract was pulverized and mixed with 1 mM of HAuCl4 in a water bath for 3 minutes at 80◦C to produce Au-NP (13). The pulp extract of Abelmoschus esculentus (L.) Moench was mixed with 1 mM of HAuCl4 at a ratio of (4:1 v/v) for 6 hours at room temperature with continuous stirring. The solution was then kept undis- turbed for further 18 hours [14]. 25 mL of 1 mM chloroauric acid (HAuCl4) were added to mL of Zingiber officinale Roscoe root extract, and the solution was heated for 20 minutes [15]. According to the findings, MPPE43 Au-NPs are non-toxic, ecofriendly, and might be used as a biomaterial in biomedical applications [16]. The use of the aqueous barkextract of Plumbago zeylanica for the production of Ag and Au-NPs is described [17]. The use of Pterocarpus santali- nus L. (Red Sanders) bark extract to facilitate the fast stable environmentally friendly production of gold nanoparticles (Au-NPs) [18]. To produce nanoparticle, extracts of Quer- cus incana leaves were used to bioreduce tetrachloroauric acid (HAuCl43-H2O) [19]. At room temperature, Au-NPs were made to utilize a Solanum nigrum (S. Nigrum) leaf extract as a reducing agent [20]. Current nanoparticle manufacturing processes are typically costly and include chemicals that may be detrimental to the environment, necessitate- ing the creation of "greener" protocols. The synthesis of AuNPs by using plant extracts is described here; it provides an alternative efficient cheap and environmental friendly technique for producing well-defined nanoparticle geometries [21]. After several in vivo experiments, the present study suggests that Au-NPs generated from the pomegranate aqueous extract could be employed as an anti-cancer therapy.

Methods and Materials

Chemicals: A peel of the pomegranate was collected from the fruit. Then, the stock solutions of trachloroaurate salt (HAuCl4) 99.98 percent) was obtained from Sigma as AuNPs. Distilled water was used to make the aqueous solu- tions and to clean and wash, all the glassware before use.

Preparing the Aqueous Pomegranate Peel Extract: The peels were first cleaned with tap water to remove dirt, rinsed with distilled water for several times, and then dried at 40◦C in a laboratory oven. They were ground and turned into a fine extract out of which 0.50 g were added to 20 ml of distilled

water and boiled at 60 ◦C for 30 minutes. Then, the solution was cooled, filtered, put in a beaker, and kept for further use.

Synthesis of Au-NPs: At 25, 80, and 100Cº, 20 ml of peel extract was put into reaction with 10 milliliters of HAuCl4 in a conical flask. Both the change in color and the time reaction took to change were noticed. The color of the solution almost instantly changes from pale brownish to red, indicating the creation of Au-NPs nanoparticles [Au/Pomegranate Pee] as shown in Figure (1) below.

The initial experiment (In-vivo experiment): The purpose of this study was to look into the effect of Photodynamic therapy (PDT) on mammary adenocarcinoma tumor growth inhibition.

Animal Model: Forty mature Swiss albino female mice weighing 20-25 grams and aged 6-8 weeks were used. At the College of Sciences/University of Babylon Animal Home, the animals were kept in well-ventilated chambers.

Transplantation of tumor cells in mice: A single tumor-bearing mouse (mammary adenocarcinoma) was provided from a prior experiment at the College of Veterinary Medicine/ University of Baghdad/ Cancer Unit. Tumor cells were collected for transplantation into mature female Swiss albino mice; the following proce- dure was used to carry out the transplanting process under extremely sterile circumstances 22:

- The tumor mass area was thoroughly cleaned with 70% ethanol.
- The contents of the tumor mass tissue were extracted using 10 ml disposable syringes, placed in a sterile flask, and suspended in 50 ml of sterile PBS.
- 1 ml of tumor cell suspension was transplanted into an adult Swiss Albino mouse (4-6 weeks old) by inserting an 18-gauge needle hypodermic anywhere between the thigh and the shoulder.

Grouping the Experiment: The animals were divided into 10 groups, with four mice in each, as follows:

- **Group (G1)** is the negative control group for mice with no mammary adenocarcinoma.
- **Group (G2)** is the positive control group for mammary adenocarcinoma-bearing mice.
- **Group (G3)** is the PDT 25% AuNPs + laser group.
- **Group (G4)** is the PDT 12.6 % AuNPs + laser group.
- **Group (G5)** is the PDT 6.25 % AuNPs + laser group.
- **Group (G6)** for tumor-bearing mice injected with I/T 25 % AuNPs.
- **Group (G7)** for tumor-bearing mice injected with I/T 12.6% AuNPs.
- **Group (G8)** for tumor-bearing mice injected with I/T 6.25% AuNPs.
- **Group (G9)** for no mammary adenocarcinoma- bearing mice received AuNPs S/C injections.
- **Group (G10)** for tumor-bearing mice exclusively treated with laser.

Administration of Photosensitizer: Mammary adenocarcinoma cells (AM3) were subcutan-eously implanted in the thigh area of female mice. After 2 weeks, the tumor has achieved the appropriate volume of at least 4.5 mm^3.

Procedure for Laser Irradiation: Measuring and calculating the wavelength were carried out by using detectors manufactured in the

laboratory to find out the energy and its distribution on the laser beam, which appeared to be Gaussian. The laser power was then measured and the intensity was extracted by using the relationship below:

$$I = {}^{P}/_{\pi r^2} = 0.009\ (W/cm^2)\ ^{(1)}$$

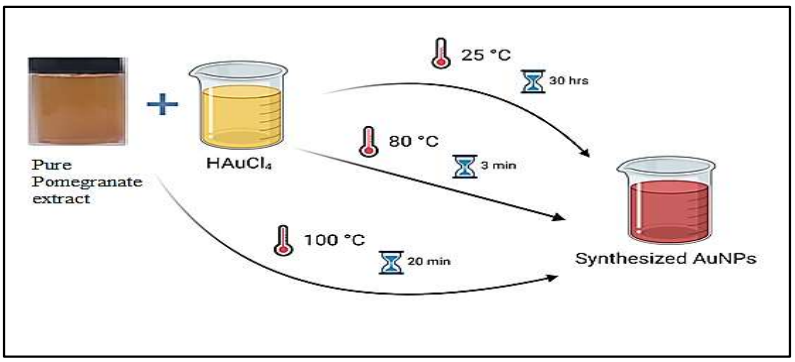

Figure 1: Green gold nanoparticle production methods (Au NPs).

Energy density (ED) is calculated from the relationship below:

$$(ED) = I\ t = 0.009\ 30 = 0.27\ Joule\backslash cm^2$$

where I=0.009(W/cm^2) and the exposure time t=30 minutes.

Statistical analysis

The computations were performed by using the SPSS statistical software for social sciences (version 19) and Mi- crosoft Office Excel (for examination of the specific signifi- cant differences among groups).

Results and Discussion

The pomegranate peel extract (0.50 g, 20 mL) acts as a re- ducing and stabilizing agent while HAuCl4 (10 mM) acts as a gold precursor. The changes in the color of the extract has shown a reduction of HAuCl4, as shown in Figure (1).

The reaction was quick as the pale brownish color of the extracts turned red within 3 min, 20 min, and 30h, show- ing the formation of Au-NPs. The chemical equations for synthesizing the Au-NPS are as follows:

$$\textbf{HAuCl4}(aq)\ \textbf{+ Pomegranate extract} \xrightarrow{Stirring\ at\ (25,80,100\ C^{\circ}\ Temp).} \textbf{Au-NPS [Au/ Pomegranate]}\ ^{(1)}$$

UV-Visible Spectroscopy Analysis: Figure (2) below shows UV-vis spectra that indicate the existence of Au-NPs. According to the findings, the pomegranate peel extracts have no discernible peak. How- ever, when HAuCl4 is added, a strong peak occurs near 593–532 mm. Other characterizations confirm that this signal implies the production of monodisperse spherical Au-NPs. Within 3min, 20min, and 30 h respectively, the reaction occurs with a noticeable color shift and (311) planes respectively; this in- dicates that Au-NPs have a face center cubic structure (fcc). By comparing the XRD pattern of Au-NPs to the

database, TCDD file number 96-901-2431 the crystallinity of Au-NPs is pure. However, there is a change in peaks with the database where pure metallic Au-NPs crystal structure is found. The Debye-Scherrer equation can be used to predict the particle size of Au-NPs.

$$d = \frac{k\,\lambda}{(\beta.\cos\theta)} \quad (1)$$

where **d** denotes the average crystallite size, **k** the Scherrer constant (0.9), X-ray wavelength (0.154 nm), line broadening in radians, and is Bragg angle.

According to the Scherrer equation, the average crystallite size of Au-NPs is 13 nm.

Transmission Electron Microscopy (TEM): The TEM was used to study the size and shape of the produced Au-NPs, as shown in Figure 4 below. The Au-NPs are well distributed with the pomegranate matrix around them showing that the pomegranate matrix functions as a capping agent to keep the Au-NPs from being aggregated. The produced Au-NPs have an average size of 19.96±0.784 nm and are primarily spherical with occasional hexagonal and triangular shapes. *X-Ray Diffraction Analysis:* The crystalline structure of the Au-NPs produced is shown in Figure 3 below by the powder X-ray diffraction pattern. The spectra show a strong peak at 2θ = 37.8227º, 43.7014º, 63.8103º, and 77.1540º which correspond to the (111), (200), (220),

Results of in vivo photodynamic therapy:

Clinical indicators include body weight, tumor volume, Tables (1,2).

A. Positive control group (G2)

The major clinical symptoms found in tumor-bearing fe- male mice were severe anorexia, resulting in gradual loss of body weight, accompanied by weakness, anemia, and cachexia, as shown in Table (1). All of these indications were observed with an increase in the tumor volume, in contrast with the negative control group (G1), as shown in Table (2) below.

B. Groups treated with PDT

In mice treated with PDT, the body weight increased within a normal range in groups (G3, G4 and G5), with no obvious clinical indications. However, the animals treated with Au-NPsshowed asubstantial dropin body weight only at the 3^{rd}, 4^{th} & 5^{th} week of experiment in comparison with (G1).

C. Groups treated with Au-NPs

There was no significant difference in the body weight of tumor-bearing mice treated with different concentra-tions of Au-NPs at the first and second weeks, i.e., immediately after the intratumoral injection of Au-NPs. Yet, there was a significant difference in body weight during the last three weeks of the experiment. Table (1) shows that G6 and G7 have a similar significant difference.

D. Group treated with laser.

There was a significant decrease in the body weight of mice in G10, treated with laser only, in comparison with G1, at the last week of experiment.

The Effect of PDT on tumor volume

A. Positive control group (G2)

Indicating that the tumor was aggressive, the tumor volume increased in a time-dependent way in G2. However, the tumor volume and a high growth inhibition percent were reduced in the groups treated with PDT, as shown in Table (2). This is due to the effects of PDT.

B. Groups treated with PDT

As the results of tumor volume shown in Table (2), tumor volume was of significant inhibition rate at level of signifi- cance ($P<0.05$). The tumor volume was decreased gradu- ally during the five weeks of experiment reaching to (0.22 mm^3) in the (G3) treated with PDT (25% Au-NPs).

C. Groups treated with Au-NPs

Only the third week after I/T delivery of Au-NPs alone did tumor volume exhibit a significant drop at a level of significance ($P<0.05$) in G6, G7, and G8. T+ 25, T+12.6, and T+ 6.25 percent of Au-NPs resulted in an increase in tumor volume to 7.06, 7.58, and 8.08 mm^3 respectively at the fifth week as shown in Table (2).

D. Groups treated with laser

Compared to G3, G4, and G5 treated diode laser (532nm) irradiation for 30 minutes without Au-NPs, Table (2) revealed a significant difference ($P<0.05$). Besides, tumor volume grew significantly in G10, reaching 8.45 mm^3 at the fifth week of the experiment, compared to 8.58 mm3 in G2 in the same week of the experiment.

Histopathological study:

A. Skin histopathology for G2.

The microscopic findings G2 showed normal epidermis and dermis with skin appendages, as seen in the histological slice of skin in Fig. (5) below.

B. Microscopic lesion of breast adenocarci- noma tumor in G2. According to the histopathological findings, the malignant mass had infiltrated the subcutaneous tissue. It was made up of acinar-like structures, trabeculae, and islands of pleo- morphic tumor cells with big hyperchromatic nuclei and a proclivity to generate enormous cells, in addition to a great number of mitotic, as seen in Figure (6) below.

C. Skin PDT group G3 histopathology:

The results of a histological study of (G3), given a 25% dose, showed that the tumor mass was replaced by fibrous connective tissue infiltrated with inflammatory cells after AuNPs + laser irradiation. Meanwhile, the epidermis displayed thickening of the prickle cell layer (acanthosis) with widespread keratin deposition, as in figures (7, 8) below.

D. Skin histopathology for PDT groups G4 and G5.

The absence of the tumor mass was replaced by fibrous connective tissue infiltrated with inflammatory cells with significant fibrosis and edema development on histological examination of group (G4) treated with 12.6 percent of AuNPs + laser irradiation, as in Figure (9).

The histological results of the skin of tumor-bearing mice treated with PDT (6.25 percent AuNPs + laser irradiation) in G5 were comparable to those in G4, with mild fibrosis and clogged blood capillaries, as in Fig (10).

E. Skin histopathology in G6, G7, and G8 groups.

The skin of tumor-bearing mice in (G6, G7, and G8) treated simply with the photosens- itizer AuNPs 25%, 12.61%, and 6.25% respectively showed comparable histological alterations, Figure (11), showing a remnant tumor mass buried in subcutaneous adipose tissue.

F. Histopathology of skin of group G9.

The major histological findings of skin in G9 tumor free mice injected with AuNPs 25% S/C include a significant sclerosis of the dermal layer with inflammatory cells

infiltration; hair follicles were dilated and infiltrated with dead neutrophils (Fig. 12,13).

G.Histopathology of skin of group G10.

The histology of skin tumor-bearing mice treated with laser alone (G10) revealed comparable results to those in G2, with the tumor mass including subcutaneous tissue. It was made up of acinar-like structures, trabeculae, and islands of pleomorphic tumor cells with big hyperchromatic nuclei Figure (14).

Table 1: The Mean ± SD of the PDT effect on the body weight (gm) of experimental groups during the five weeks of experiment.

Groups	1 st week	2 nd week	3 rd week	4 th week	5 th week
G1	19.67 ±1.08	19.6±1.08	20.1 ± 1.10	21.11± 1.15	22.2 ±1.08
Control -ve	A	A	a	a	a
G2	18.90 ±1.15	18.21±0.84	17 .1± 1.09	17 .81± 1.09	17.23 ±0.51
Control +ve	A	A	b	b	b
G3	19.1±2.08	19.78±1.71	20.1±1.68	21.01±1.68	22.5 0±1.41
PDT 25% AuNPs	A	A	a	a	a
G4	18.7±3.10	19.68±2.91	20.08 ±2.37	21.08 ±2.37	22.6 ±2.96
PDT 12.6 % AuNPs	A	A	a	a	a
G5	18.17±1.32	19.6±1.32	20.6 ±1.32	20.6 ±1.32	20.25 ±2.25
PDT 6.25 % AuNPs	A	A	a	a	a
G6	18.07±1.28	18.6 ±0.87	16.53±0.87	16.53 ±0.87	16.11 ±0.49
T+25% AuNPs	A	A	b	*b*	b
G7	19.08±3.16	18.33±3.18	16.83 ±3.18	16.83± 3.18	16.83 ±2.22
T+ 12.6 % AuNPs	A	A	b	b	b
G8	22.33±4.92	21.33±4.92	21.16 ±4.44	21.16 ±4.44	20.66 ±3.20
T+ 6.25 % AuNPs	A	A	a	a	a
G9	19 ±2.36	19.75±2.38	20.5 ± 2.10	20.5 ± 2.09	22.66 ±0.98
S/C AuNPs	A	A	a	a	a
G10	18.67±2.60	18.63±2.02	18.05 ±1.15	18.05 ±1.15	16.83 ±0.98
T+L	A	A	a	a	a

Tukey's test revealed that the mean values of body weight (gm) followed by distinct letters differed substantially ($P<0.05$) across experimental groups

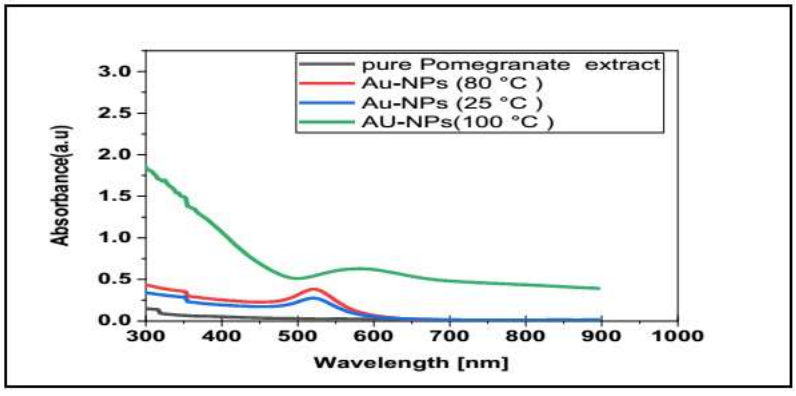

Figure 2: UV-Vis spectroscopy graph of synthesized Au-NPs. Pomegranate peel extracts.

Table 2: The Mean ±SD of the tumor volume (mm^3) for the experimental groups during the course of the five-week trial.

Groups	0	1 st week	2 nd week	3 rd week	4 th week	5 th week
Control +ve	4.74 ± 1.20 a	5.57 ± 0.46 a	6.85 ± 1.07 a	7.81 ± 1.03 A	8.31 ± 0.41 a	8.58 ± 0.72 a
PDT 25% AuNPs	4.79 ± 1.21 a	1.31 ± 0.54 b	1.65 ± 0.55 bc	1.39 ± 0.52 bcd	0.87 ± 0.77 b	0.22 ± 0.58 bc
PDT 12.6 % AuNPs	4.79 ± 0.18 a	2.23 ± 0.21 b	3.12 ± 0.68 b	2.37 ± 0.63 bcd	1.85 ± 0.37 bc	1.11 ± 0.83 bc
PDT 6.25 % AuNPs	4.30 ± 0.17 a	3.31 ± 0.37 b	3.73 ± 0.53 b	2.84 ± 0.68 bc	2.43 ± 0.36 bc	2.6 ± 0.81 b
T+ 25% AuNPs	4.47 ± 0.20 a	5.21 ± 0.60 a	5.63 ± 0.60 a	6.01 ± 0.51 b	7.23 ± 0.41 a	7.06 ± 1.30 a
T+ 12.6 % AuNPs	4.23 ± 0.28 A	4.85 ± 0.50 a	5.53 ± 1.05 a	5.88 ± 0.91 b	7.10 ± 0.56 a	7.58 ± 0.94 a
T+6.25 % AuNPs	4.91 ± 0.30 a	5.51 ± 0.61 a	5.91 ± 0.72 a	6.38 ± 0.73 b	7.41 ± 0.75 a	8.08 ± 0.56 a
T+L	4.55 ± 0. 35 a	5.47 ± 0.54 a	6.70 ± 0.87 a	7.58 ± 0.84 a	8.18 ± 0.40 a	8.45 ± 0.95 a

Tukey's test revealed that mean ±.SD values followed by various tiny letters differed substantially (P<0.05) across experimental groups.

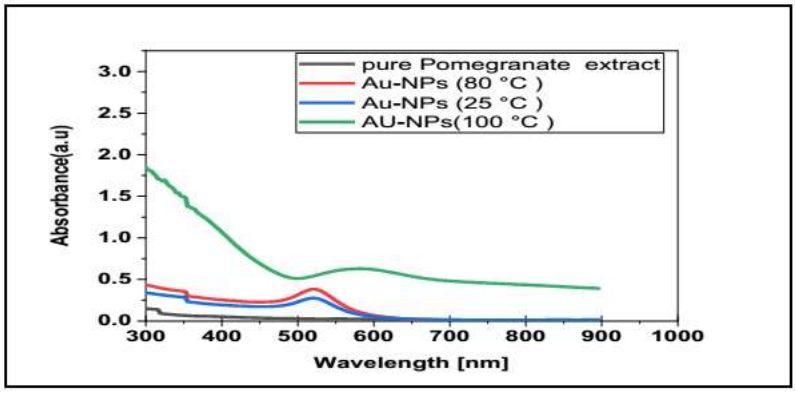

Figure 2: UV-Vis spectroscopy graph of synthesized Au-NPs. Pomegranate peel extracts.

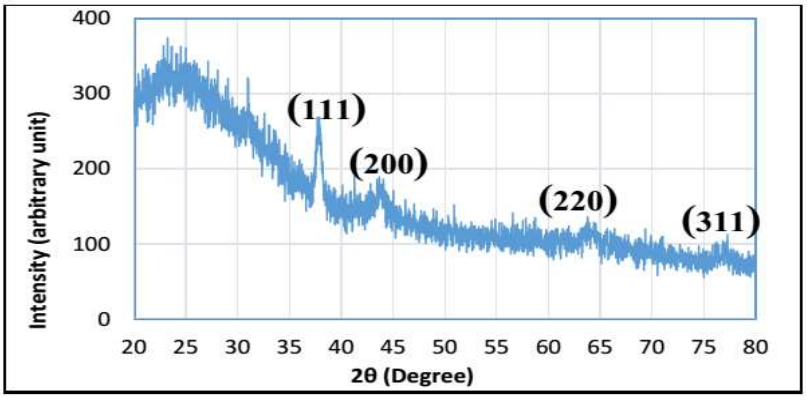

Figure 3: XRD spectra of Au-NPs prepared by pomegranate peel extracts with reference peak's intensity.

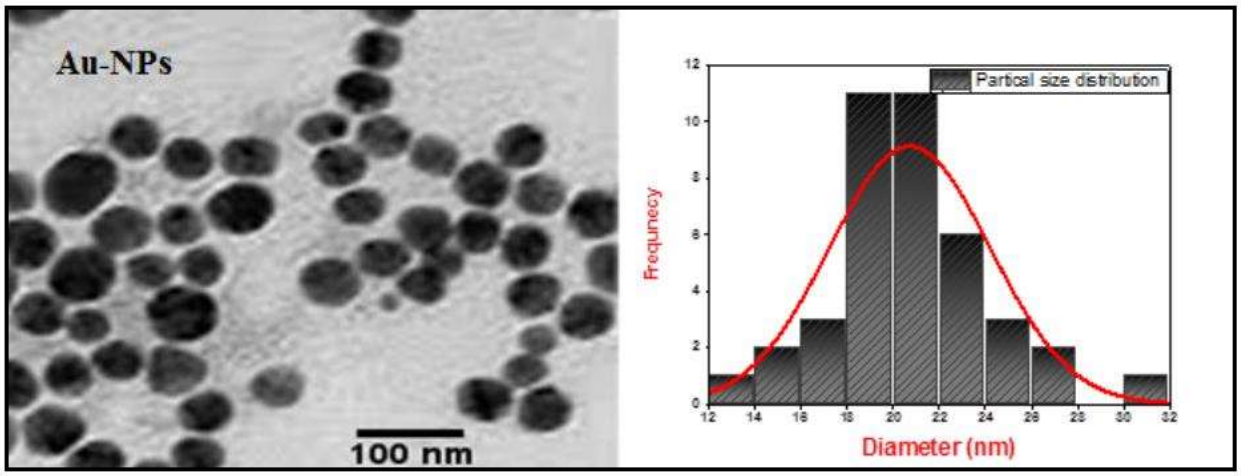

Figure 4: TEM image of Au-NPs formed by pomegranate peel extracts and the histogram of the particle size distribution of Au-NPs with ×250000 magnification power.

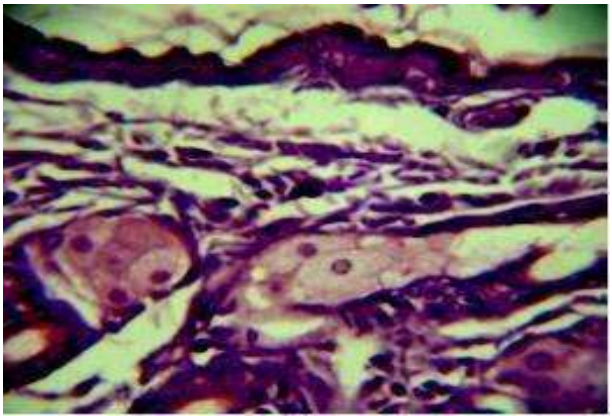

Figure 5: A Normal histological structure of mouse skin in a histological section, with power of magnification (400 X) control (-ve) group (G1).

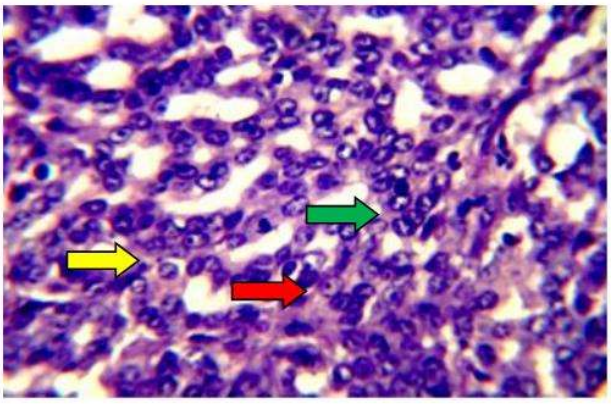

Figure 6: A histopathological section of a tumor mass in the skin of a tumor-bearing female mouse (control+ve group G2), demonstrating the growth of pleomorphic tumor cells ( ) with hyperchromatic nuclei ( ) & a large number of mitotic figures ( ) (H&E stain with power of magnification 400x).

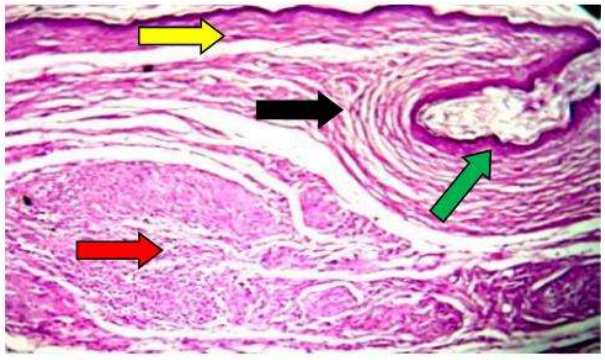

Figure 7: A histopathological segment of mouse skin (PDT group G3) exhibiting extensive fibrosis ( ) with inflammatory cell infiltration ( ) replacing the tumor mass with hyperkeratosis ( ) & acanthosis ( ) (H&E stain with power of magnification 100X).

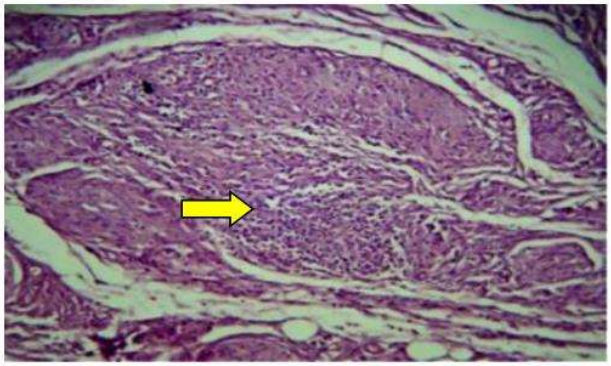

Figure 8: Fibrosis and inflammatory cells infiltration ( ) replacing the tumor mass in a histopathological segment of mouse skin (PDT groupG3) (H&E stain with power of magnification 400 X).

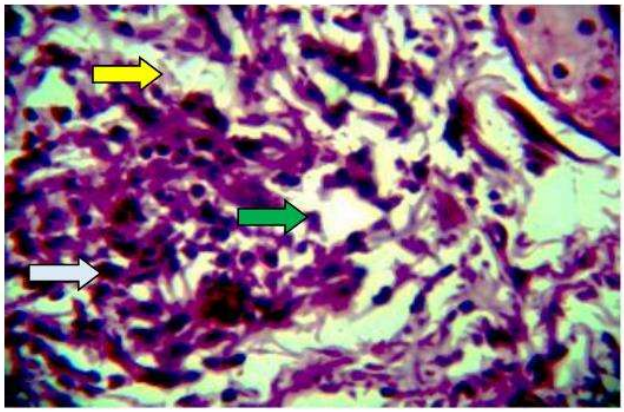

Figure 9: A Histopathological section of mouse skin in G11demonstrating a moderate fibrosis ( ) with inflammatory cell infiltration ( ) and edema formation ( ) (H&E stain with power of magnification 400 X).

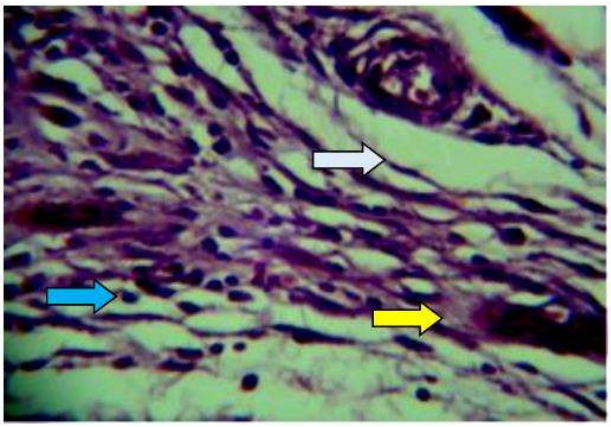

Figure 10: A Histopathological section of skin of G12 showing slight fibrosis (⇨) with few inflammatory cells infiltration (➡) with congested blood capillaries (➡) (H&E stain with power of magnification 400 X).

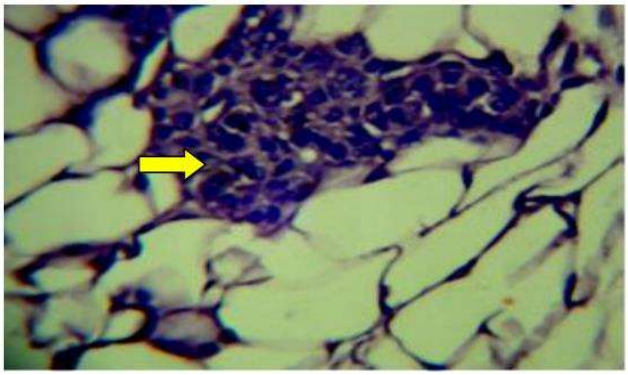

Figure 11: A histopathological section of tumor mass in the skin of tumor-bearing female mouse treated with 25% AuNPs only without laser (G6) showing remnant of tumor cells (➡) within adipose tissue (H&E stain with power of magnification 400 X).

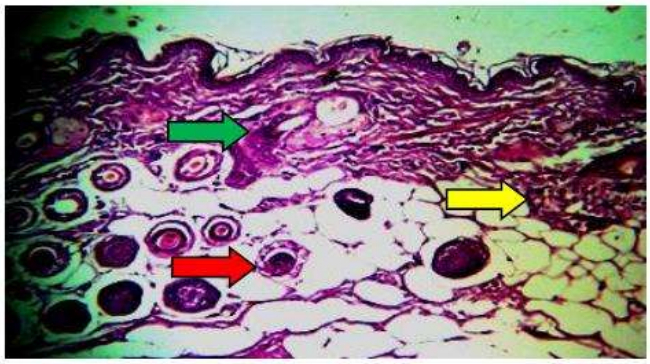

Figure 12: Sclerosis of the dermis (➡) with inflammatory cell infiltration (➡) with folliculitis (➡) (H&E stain with power of magnification 100 X).

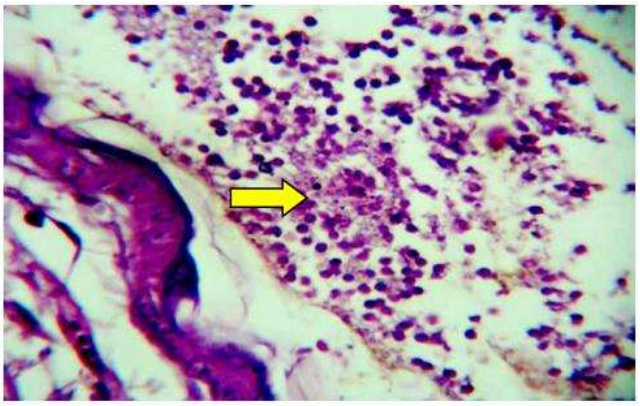

Figure 13: A histological section of mouse skin from G9 showing aggregations of large numbers of neutrophils on the surface (⇨) (H&E stain with power of magnification 400 X)

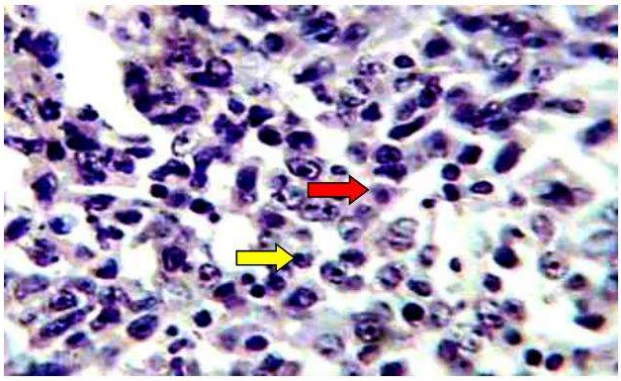

Figure 14: A histopathological section of tumor mass in skin of tumor-bearing female mouse in G10 showing the proliferation of pleomorphic tumor cells (⇨) with hyperchromatic nuclei (⇨) (H&E stain with power of magnification 400 X).

Conclusions

This study evaluated the anticancer effects of Au-NPs cre- ated by reducing aqueous gold metal ions in the presence of aqueous pomegranate peel extract. The study aimed to evaluate the photodynamic treatment using a laser with a wavelength of 532 nm with green gold particles as a pho- tosensitizer in vivo experiments.

Increasing the exposure duration to 30 minutes by us- ing a diode laser with an ED of 0.27 Joule/cm^2 after in jecting 25% of Au-NPs intramurally resulted in a com- plete elimination of tumors in mammary adenocarcinoma- bearing mice. After injecting 25% of Au-NPs into mammary adenocarcinoma-bearing mice, histological investigations indicated the development of anti-tumor immunity after 30 minutes of irradiation with a diode laser with an ED.

Biologically produced NPs can also be used in agriculture as an alternative to pesticides and herbicides, nd to remove any hazardous microorganisms from water as well.

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Conflict of interest

The author states that she has no competing interests with respect to the publication.

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Factors Affecting the Transmission of Hepatitis C Among the Thalassemic Patients in Holy Najaf

Hassan Musa Abd [(1)], Mohammed Naji Atiyah [(2)], Mohammed Majeed Faisal [(3)], Alaa Jumaah Manji Nasrawi [(4)], Ali Hussain Al-faydawi [(5)]

[(1)] Senior Pediatrician, Karbala Health Directorate, Iraq, [(2)] Senior Pediatrician, Department of Pediatrics, Karbala hospital for Children, Hereditary Blood Disease Center, Karbala, Iraq, [(3)] Senior Pediatrician, Al Najaf Health Directorate, Iraq., [(4,5)] Professor of Pediatrics, University of Kufa, Faculty of Medicine, Iraq

Abstract

On a global scale, thalassemia is the most frequent genetic condition. The reason that has made thalassemia so widespread are unknown, but they are thought to be related to malaria's geographic distribution. Children with thalassemia have shorter red cell lives, fetal hemoglobin in their red cells longer than normal, and more susceptible red cells to oxidative stress. In humans, thalassemia major is a prevalent hemoglobinopathy. Because thalassemia patients require multiple blood transfusions which is a common transmission vector for hepatitis C virus (HCV), numerous studies have found varying prevalence of hepatitis C among thalassemia major patients. As a result, this study was carried out to discover anti-HCV in thalassemia patients in our location. The main aim of this study is to find out the factors that increase the chance of getting HCV infection in thalassemia patients via a clinical and serological investigation of those patients in Holy Najaf. This is a descriptive analytic study carried out at The Hematology Center in al–Zahraa Teaching Hospital for Maternity and Children in Holy Najaf from October 2019 to October 2020. A total number of 550 patients, 330 males and 220 females, who registered in thalassemic center were surveyed; among them, 48 thalassemic patients were found to have HCV infection when their medical records were analyzed. A detailed clinical account was made for every patient by using a proforma, taking into account information like name, age, sex, residency, age at starting blood transfusion, frequency of blood transfusion per month, splenectomized or not, blood group, HCV antibodies test, and liver function test. The total infected donors with HCV were 43 (0.17%) and 62 (0.30%) at 2019 and 2020 respectively. The study group comprised 48 thalassemic patients, 33 (68.75%) males and 15 (31.25%) females. The mean ± SD age was 18.4 ± 7.57months ranging from 2 to 35 years old. There was a direct association between age and seropositivity to anti-HCV as the latter was significantly associated with older ages ($P \leq 0.01$). However, there was no association between gender and anti-HCV seropositivity as there were no significant differences ($P= 0.653$). Out of 48 patients, 26 (54.14%) live in urban while 22 (45.17%) live in rural areas. In regard to residency, however, there was no direct relationship ($P \leq 0.01$) between the residency and the seropositivity to anti-HCV. Yet, there is a high significant relationship ($P \leq 0.01$) between frequency of blood transfusion per month and seropositivity to anti-HCV. HCV seropositivity was significantly associated with the longer duration of the disease ($P \leq 0.01$). Besides, patients of O+ blood group represented a higher ($P \leq 0.01$) seropositivity to anti-HCV than in patients with other blood groups; meanwhile, patients with Rh- showed a lower degree of seropositivity to anti-HCV. In addition, the study showed a highly significant ($P \leq 0.01$) relationship between splenectomy and seropositivity to anti-HCV.

Key words: β- thalassemia; Hepatitis C; Seropositivity.

INTRODUCTION

Thalassemia syndromes are inherited hemoglobin synthesis abnormalities in which one or more globin chains are produced insufficiently or not at all. Alpha-thalassemia and beta-thalassemia are the two most frequent kinds of thalassemia, in which one of the two chains is formed in insufficient amounts [1].

Beta-thalassemia major is said to be prevalent predominantly in the Mediterranean and Middle Eastern region, including Italy, Greece, and Turkey, and Iran, Pakistan, India, and China, respectively. In these countries, the frequency of the thalassemia gene ranges between 5 and 25%. In the Far East, notably Thailand, alpha-thalassemia is common, with about one-fourth of the population carrying the gene for one of the two types of thalassemia[2].

The name of the globin whose production is affected is used to classify thalassemia genetically. There is an excess of alpha chain synthesis compared to beta chain synthesis in beta-thalassemia disorders. The abnormalities are expressed in both heterozygous and homozygous individuals, and the inheritance mechanism is autosomal. Beta thalassemia is divided into two kinds, each with further subclasses. Thalassemia minor, or trait, refers to heterozygous state, whereas thalassemia major, or Cooley's anemia, refers to homozygous state. A multitude of factors, including race and interaction with other inherited erythrocytic illnesses, determine the severity of certain kinds of thalassemia[3]

HCV infection is seen all over the world and according to World Health Organization (WHO) estimates, up to 3% of the world's population (170 million) is infected with HCV[4]. Children with thalassemia who undergo repeated blood transfusions are at an increased risk of contracting the HCV[5]. Infection rates in healthy blood donors range from 0.01 to 0.02% in northern Europe, 1 to 1.5% in southern Europe, and 6.5% in portions of equatorial Africa[6]. In Egypt, prevalence rates have been reported to be as high as 20%. Because of this disparity, alternative preventive techniques, community intervenetions, and even therapy procedures must be chosen based on economic and social factors [7].

Only 15-25% of sick people appear to have totally recovered from their infection and are no longer contagious. This implies the rest develop chronic infection and may become contagious as soon as one to two weeks after exposure, and they will remain infectious (carrier) for the rest of their lives. Approximately 30% of chronically infected people just have persistent infection and do not develop liver disease, refed them as "healthy carriers"; 50% have no symptoms but have elevated liver enzymes, and 20% have clinical liver disease [8]. A chronic liver disease may progress to cirrhosis in between 10 to 38% within approximately 20 years while a hepatocellular carcinoma develops within approximately 10 years after appearance of cirrhosis, with a prevalence of at least 20% [9].

Diagnosis

1.Serological tests for HCV specific antibodies: An anti-HCV antibody is not protective; it does not confer immunity, and is usually present simultaneously with viruses [10].

(a) Enzyme Immuno Assay (EIA)

HCV is usually positive for approximately 6-8 week after the patient's exposure to it, whether or not having symptoms. Meanwhile, the sensitivity of EIA is at least 95% after window period.

(b) Recombinant Immuno Blot Assay (RIBA)

RIBA procedures are carried out on a cellulose strip, where four HCV antigens are blotted and reacted with the serum of the patient. In 85% of HCV-infected patients who have reactivity in at least two bands (RIBA-2), results are observed to be positive.[11]

2. Direct demonstration of HCV RNA by PCR: A reverse transcriptase converts viral RNA from whole blood, serum, plasma, and fixed tissues to complementary DNA (cDNA), which is then amplified by polymerase chain reaction (PCR). This is a confirmatory test that is both sensitive and specific and is particularly useful for monitoring the efficacy of interferon therapy.[8] False negative, but not false positive, results do exist.

3. Quantification of HCF RNA: The test results may be used to distinguish between treatment responders and nonresponders[9].

4. Determination of HCV genotypes: The diagnosis of HCV genotypes will also aid in determining the infection's severity, potential prognosis, and management. Type Ib appears to be more aggressive, poorly reacting to interferon therapy, and necessitating more liver transplants, whereas type 2 appears to be more benign and responds well to interferon therapy[12,13].

Treatment

For six months, a three-time a week interferon Alfa-2b (Intron A) 3 million U subcutaneously is the conventional treatment. This therapy produces an initial response in 40% of patients, however, only half or less of these individuals have a longterm maintained response. In this therapy, what influences the response of chronic hepatitis C is the viral genotype, Ib type with poor response, level of viremia, severity of liver disease, and hepatic iron content, or high serum ferritin level mien poor response [14].

This study aims to show the possible risk factors of getting infection with HCV in thalassemic patients in Holy Najaf.

Materials and Methods

This study was carried out at the hematology center in al-Zahraa Teaching Hospital for Maternity and Children in Holy Najaf from October 2019 to October 2020 where a total of 550 patients registered in the Thalassemic Center were surveyed and the medical records of 48 thalassemic patients infected with HCV were analyzed.

The data collected from each medical record includes:

1- Name, age, sex and residence.
2- Age at starting blood transfusion.
3- Frequency of blood transfusion per month
4- Splenectomies or not.

The total number of blood donors and the patients infected with HCV during 2019-2020 in Holy Najaf were collected from the Public Health Department, CDC Section, in Najaf Health Directorate. Then, in a test for HCV antibodies, a second-generation ELISA kit was used for identifying HCV antibodies. The instrument used was Biotech, manufactured in China and the procedure for detecting HCV was made by Foresight method.

As for the liver function tests, 5 ml of blood sample were collected from each patient; 200 micro serums were analyzed.

The three enzymes of ALT, AST and ALP were assessed on the Chemistry autoanalyzer (bt. 35i), manufactured in Turkey.

All of the analyses were statistical carried out with software (SPSS version 21). The Chi squared (X2) test was used to look for the significant differences in nonparametric data (tables 2–8). Besides, the paired sample t-test was used to evaluate the continuous variables in Table 9 (enzymes assay). At 1% and 5%, P-values of 0.05 and 0.01 were considered statistically significant and highly significant, respectively.

Results

Table (1) above shows that the total infected donors with HCV were 43 (0.17%) and 62 (0.30%) at 2019 and 2020 respectively. The total number of thalassemic patients was 550. The study group comprised 48 anti-HCV seropositive thalassemic patients (33 [68.75%] males and 15 [31.25%] females). The mean ± SD age in months was 18.4 ± 7.57 (range: 2 – 35 months).

Table (2) below shows the association between age and seropositivity to anti-HCV; there is a direct association between the age and the seropositivity to anti –HCV where HCV seropositivity was significantly associated with an older age ($P \leq 0.01$).

However, Table (3) below demonstrates no association between gender and seropositivity to anti –HCV where there are no significant differences ($P = 0.653$).

Table 1: Total number of blood donors and percentage of HCV infections among them during 2019-2020 in Holy Najaf

Month	2010			2011		
	No. of Blood donors	Infected		No. of Blood donors	Infected	
		No.	%		No.	%
Jan.	1786	-	0	2210	11	0.50
Feb.	1780	8	0.45	1486	11	0.74
Mar.	2122	5	0.24	2148	2	0.09
Apr.	2025	4	0.20	1918	-	0
May.	2991	5	0.17	2113	2	0.09
Jun.	2235	2	0.09	2153	13	0.60
Jul.	1996	14	0.70	2408	17	0.71
Aug.	2075	-	0	1657	1	0.06
Sep.	1613	-	0	1280	3	0.23
Oct.	1929	2	0.10	2029	1	0.05
Nov.	1789	1	0.06	2879	1	0.03
Dec.	2175	2	0.09	2133	11	0.52
Total	24516	43	0.17	24414	62	0.30

Table 2: Association between age and seropositivity to anti -HCV

Age in year	No.	(%)	P-value
< 5	7	14.58	
≥ 5 – 10	8	16.67	
> 10 – 15	10	20.83	0.000**
>15 – 20	12	25.00	
>20	11	22.92	
Total	48	100	

** *Significant at P* ≤ 0.01, Chi squared (X^2) test

Table 3: Association between gender and seropositivity to anti -HCV

Gender	No.	%	P-value
Male	25	52.08	0.653 NS
Female	23	47.92	
Total	48	100	

NS=no significant at P ≤ 0.05, Chi squared (X^2) test

Table (4) below demonstrates that out of 48 patients, 26 (54.14%) live in urban, 22 (45.17%) in rural area. In regard to residency, there is no direct association (*P* ≥ 0.05) between the residency and the seropositivity to anti-HCV.

According to Table (5) below, there is a significant correlation between blood transfusion per month and that making blood transfusion more than once a month was highly affecting (62.5%) than that of once a month in the other group.

Table (6), on its turn, shows that there is a direct relationship between duration of the disease and the seropositivity to anti-HCV, where HCV seropositivity was significantly associated with the longer duration of the disease (*P* ≤ 0.01).

Patients of O+ blood group represented the higher (*P* ≤ 0.01) seropositivity to anti-HCV than those of other blood groups, *cf.* Figure 4. However, patients with Rh-ve showed lower seropositivity to anti-HCV, as shown in Table (7) below.

Table (8) shows that there is a highly significant (*P* ≤ 0.01) associa-tion between splenectomy and the seropositivity to anti-HCV, 28 (58.33%) patients with splenectomy had positive hepatitis while 20 (41.67%) non-splenectomized patients were hepatitis positive.

Table (9) shows the liver enzymes in previous and recent periods of infection, the titer of all liver enzymes was higher in recent (*P* ≤0.05) than previous periods.

Table 4: Association between residence and seropositivity to anti -HCV

Residence	No.	%	P-value
Urban	26	54.17	0.092 NS
Rural	22	45.83	
Total	48	100	

NS=no significant at P ≤ 0.05, Chi squared (X^2) test

Table 5: Association between blood transfusion per month and seropositivity to anti-HCV

No of Blood Transfusion	No.	%	P-value
Once per month	18	37.5	0.000**
More than once per month	30	62.5	
Total	48	100	

** *significant at P* ≤ 0.01, Chi squared (X^2) test.

Table (6). Association between duration of disease and seropositivity to anti-HCV

Duration of disease	No.	%	P-value
<1 yr	5	10.42	
1 – 5	16	33.33	0.006**
>5	27	56.25	
Total	48	100	

** *significant at P* ≤ 0.01, Chi squared (X^2) test.

Table 7: The Association between blood groups and seropositivity to anti-HCV

Blood groups	No.	%	P-value
A+	10	20.83	
A-	1	2.08	
B+	10	20.83	
B-	1	2.08	
AB+	8	16.67	0.001**
O+	16	33.33	
O-	2	4.16	
Total	48	100	

** *Significant at P* ≤ 0.01, Chi squared (X^2) test

Table 8: The Association between splenectomy and seropositivity to anti-HCV

Splenectomy	No.	%	P-value
Splenectomized patients	28	58.33	
Non-splenectomized patients	20	41.67	0.000**
Total	48	100	

** *significant at P* ≤ 0.01, Chi squared (X^2) test

Table (9) Liver enzymes in previous (six months) and recent period of infection

Liver enzymes	previous	recent	P-value
SGpT(ALT)	44.74±3.49	49.87±4.86	0.034*
SGoT(AST)	32.40±3.75	39.25±4.32	0.032*
ALP	84.62±11.69	110.22±13.860	0.013*

* *Significant at P* ≤ 0.05, t-student test.

Discussion:

The risk of hepatitis infection among thalassemic patients is related to many factors that have important effects on the prevalence of hepatitis infection. These factors include age, gender, residence, frequency of blood trans-fusion per month, blood group, duration of thalassemia and splenictomy [15].

The present study has shown that in Najaf hematology center, the total donors infected with HCV were 43 (0.17%) and 43 (0.30%) in 2019 and 2020 respectively. By contrast, other studies showed that infection rates among healthy blood donors range from 0.01-0.02% in northern Europe, 1-1.5% in Southern Europe, and 6.5% in portions of Equatorial Africa [16].

This study has revealed that 8.73% of thalassemic patients were infected with HCV. However, another study Mosul Province (Iraq) reported that 26.20% of thalassemic patients were infected with HCV[17]. A higher infection rate (18%) was reported in Iran [15]. The other studies from some neighboring Arabic countries reported an HCV infection rate of 33% in Kuwait in1998 (sample size 129 patients) [18] and 40% in Bahrain in 1995 (sample size 242 patients) [19] and in Jordan in 2001(sample size 143 patients) [20].

A study by Allavian et al. (2010) compiled all available data on epidemiological characteristics and risk factors affecting HCV infection in thalassemia patients in Eastern Mediterranean countries. They came to the conclusion that the results of the available study in this area are highly disparate, and the prevalence of HCV infection among the individuals living in this area is still unknown. [15] On the contrary, the present study has shown that the prevalence of hepatitis C was significantly higher among the older age group because this group usually has a longer duration and frequency of transfusion. Similarly, HCV infection was found to be highly prevalent in the older age groups of Taiwan thalassemic patients [21].

In this study, gender and residency were not risk factors for HCV. However, Abdul Nasir [22] statistically found in the region of

Southern Punjab there was significant evidence of gender survival that male patients of Thalassemia are at a higher risk than female patients. Besides, for Ali et al, males had a higher prevalence (17.30%) of HCV than that (12.68%) in females and that males are more likely to have HCV since they are exposed to more risk factors [23].

Furthermore, the present study has shown an evident association between the frequency of blood transfusion and prevalence of hepatitis C. It has been found that 37.5% of thalassemic patients who transfused once per month are hepatitis positive while 62.5% of those who received transfusion more than once per month were positive for hepatitis C. Yet, for Angelucci and Pilo (2008) [24], the prevalence of HCV infection was demonstrated to be related to the number of units of blood received among thalassemic patients transfused before the 1990s, and surpassed 80% in adult patients.

The rate of new infections in thalassemia patients has decreased significantly in recent years in nations with a high Human Development Index, but this has not been the case in countries with a low-medium Human Development Index. This was proved by unpublished data from an Androulla Eleftheriou survey done on behalf of the Thalassemia International Federation (TIF) from 2005 to 2007. HCV infection is a significant problem for patients with thalassemia major because the vast majority of continuously transfused patients live in underdeveloped or developing countries.

Shahram Mirmomen [25], followed the commencement of blood donor screening in Iran in 1995, demonstrated that the prevalence of HCV infection reduced considerably from 22.8% to 2.6%. Furthermore, patients who received unscreened blood were exposed to HCV infection six times more than those who received blood after the screening program began. The rigorous screening of blood donors is likely to eliminate the incidence of HCV infection among thalassemic patients in Iran in the future. He stated that these findings strongly suggested that blood transfusion was the most common cause of HCV infection in thalassemic patients, and that donor screening was extremely effective in preventing or mitigating viral transmission. The efficiency of blood donor screening has also been established in cohort studies from Italy [26] and the United States [27].

The higher rate of HCV infection in older patients, patients with thalassemia major, and subjects with higher serum ferritin levels indicating that more units of blood were transfused, highlighted the importance of providing safe blood to reduce the incidence of HCV infection in the thalassemic population [27].

In addition, the present study has shown that there is a direct association between the duration of the disease and the seropositivity to anti-HCV as HCV seropositivity was significantly associated with the longer duration of the disease ($P \leq 0.01$). This goes in agreement with other studies [27].

The prevalence of hepatitis C among the patients with O blood group was more than the other patients; this is maybe due to blood group O is higher than other group, therefore, it seems that they are at higher risk of infection than other patients.

This data is consistent with those of Ansari (28), who found that patients with the O blood type had the highest prevalence of hepatitis C (20.6%), implying that this blood group is more susceptible to contamination than other blood groups. As a result, proper screening of this blood type is much more critical.

Liver enzymes showed that Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) levels significantly increased in recent reading of patients as compared to their previous levels. Touran S. [29] observed that a link was discovered between HCV RNA PCR and abnormal liver testing. The findings were in line with a recent study in Tonekabon thalassemic patients, which found a link between increased ALT and iron overload, transfusion index, age, and anti-HCV positive. Even in the absence of HCV infection, aminotrans-ferase flares are common in thalassemic patients, according to long-term follow-up of these patients. The levels of AST, ALT, and ferritin dramatically changed in patients, according to Touran S. [29] who hypothesized that there was a correlation between ALT and AST, ferritin and age, and ALT and ferritin.

Asma, et al [30] stated that serum ALT and AST more significantly raised in HCV positive patients in comparison to other thalassemic. Shekhar, et al [31] found that the Alanine aminotransferase Aspartate aminotransferase and Alkaline phosphatase levels significantly increased in posttrans-fused patients as compared to their pre-transfused levels.

Anti-HCV antibody has been linked to a number of risk factors. Anti-HCV positive was linked to the number of blood transfusion units, splenectomy, and thal-assemia duration in thalassemic patients [32].

In the present study, 50% of Splenectomized patients had positive hepatitis while 25% of the non-splenectomized patients were hepatitis positive. Splenectomy acts as an important risk factor. This is because many of Splenectomized patients are older than non-splenectomized patients (i.e., additional risk factor), and splenic-tomy done mostly in thalassemic patient because of hypersplen-ism which in turn increase the number of blood trans-fusion and the risk of hepatitis. So, early splenectomy (after 4 years and before 15 years) with good preoperative management can decrease the risk of hepatitis infection by decreasing the need for blood transfusion, with no significant increase in the rate of postoperative morbidity and mortality [33].

CONCLUSION

1- The percent of HCV among thalassemic patients in Holy Najaf is lower than previous Iraqi studies.
2- Seropositivity is significantly related to age, frequency of blood transfusion per month, duration of the disease, and splenectomy.
3- Gender and residency show no associa-tion with the prevalence of HCV.
4- Patients of O+ blood group represented the higher seropositivity to anti-HCV than patients with other blood groups.

RECOMMENDATIONS

1- In thalassemia patients with HCV chronic hepatitis or cirrhosis who have contraindica-tions to antiviral medication or have previously failed antiviral therapy, clinical surveillance of liver disease is required.
2- Effective screening program should be applied to all the donor of blood and blood product. It should have more than one screening available to increase its sensitivity as much as possible; so, further ensuring of the safety of donated blood, like use of PCR in screening program, is needed.
3- HCV infected donor should be followed; their families especially their partners should be followed as well by screening.

4- Further studies are necessary to prove if there is factual relationship between blood group & hepatitis C in thalassemic patients.

5- Patients who have been infected with HCV for a long time need to be counseled on how to avoid transmitting the virus to others.

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Late Presentation of Partial Atrioventricular Septal Defect: A Case Report

Ghada Kareem [1], Wadhah Mahbuba [2]

[1] Medical Doctor, Diwaniyah, Iraq, [2] Department of Surgery, Faculty of Medicine, University of Kufa, Iraq.

Corresponding author, ghada_nayef@yahoo.ie

Abstract

Atrioventricular septal defect is a spectrum of congenital heart defects that can be classified as either complete or partial with the complete form being the most common and usually present in the neonatal period with congestive heart failure. However, partial defects can remain asymptomatic and present later in adulthood with variable degrees of heart failure. The case under investigation is a middle aged female with a 2-year history of worsening dyspnea and recurrent chest infections due to undiagnosed partial atrioventricular septal defects. As a conclusion, it is important to establish a screening program to detect the asymptomatic cases early and prevent delay consequences.

Key words: partial atrioventricular septal defect, adult, screening

Background

Atrioventricular septal defect (AVSD) is a spectrum of congenital heart diseases that can have varying degrees of severity ranging from a clinical presentation of congestive heart failure in the neonatal period to a subtle disease that can present later in life and with less severe symptoms, depending on the size and nature of defect [1]. It results from a congenital defect in the endocardial cushion which is responsible for atrial and ventricular septation and formation of atrioventricular (AV) valves [2]. Such a defect can be complete, i.e., ostium primum atrial septal defect (ASD), common AV valve and inlet ventricular septal defect, or partial [3]. Partial AVSD (pAVSD) can be defined as ostium primum ASD and a cleft in the anterior mitral leaflet[4]. AVSD constitutes around 5-8% of the estimated cardiovascular malformations with the complete form most commonly linked to major chromosomal abnormalities and Down syndrome being on top. On the other hand, pAVSD is more common in patients without Down syndrome [5,6].

This report presents a case of 52 years old female who came to the outpatient clinic complaining, for 2 years duration, of shortness of breath on exertion. After clinical examination and routine investigation including a transthoracic echocardiography (TTE), she was diagnosed with large ASD primum, clefted anterior mitral valve leaflet with mitral and tricuspid regurgitation. Surgery was done to repair the defect and post-operative period was uneventful.

The decision to share this case was to discuss the importance of establishing a

screening method for congenital heart diseases and to draw attention to the fact that congenital heart diseases should be kept in mind as part of the differential diagnosis of dyspnea even in older age groups.

Case presentation

A 52-year old diabetic and hypertensive housewife, height: 145 cm, weight: 61 kg, presented to an outpatient clinic with a history of shortness of breath on minimal activity (NYHA class III) associated with orthopnea. Two years before her presentation, her condition started as chest infection that led to a slow progressive feeling of tiredness and shortness of breath with activity. The patient was completely well and fit before this episode with very good exercise tolerance. She reported worsening of symptoms with time and associated recurrent chest infections, palpitation and chest pain. She sought medical attention and general and cardiorespiratory examinations that showed pansystolic murmur at mitral and tricuspid areas and coarse crackles at both lung bases. On further questioning, the patient admitted having abdominal discomfort; examination showed grade 1-2 pitting lower extremity edema. Electrocardiography (ECG) showed a PR interval prolongation (240 ms) and left axis deviation, findings consistent with her later diagnosis of pAVSD [7] (Fig. 1). Chest x- ray showed an increased cardiothoracic ratio with pulmonary congestion (Fig. 2A). TTE showed a large ASD primum with dilation of the left and right atria, right ventricle, and normal ejection fraction with mitral and tricuspid regurgitation (Fig. 3). Hence, the patient was referred for coronary and cardiac chamber catheterization. While coronary angiography was completely normal, cardiac chamber catheterization showed a pulmonary artery systolic pressure of 10 mmHg. Open cardiac surgery was accordingly planned, see details in Intraoperative notes; Figure (2B) shows postoperative chest x- ray.

As a follow up for the patient, an appointment was done after one month of surgery and the patient was recovering very well; TTE showed no residual mitral regurgitation, closed ASD with no leak and good left ventricular function. After 3 months, the patient was in good health with no reported complains.

Preoperative and surgical interventions

In the preoperative period, all necessary biochemical and serological investigations were done; findings are shown in Table (1). ECG and Echocardiography were repeated and showed the same results as before.

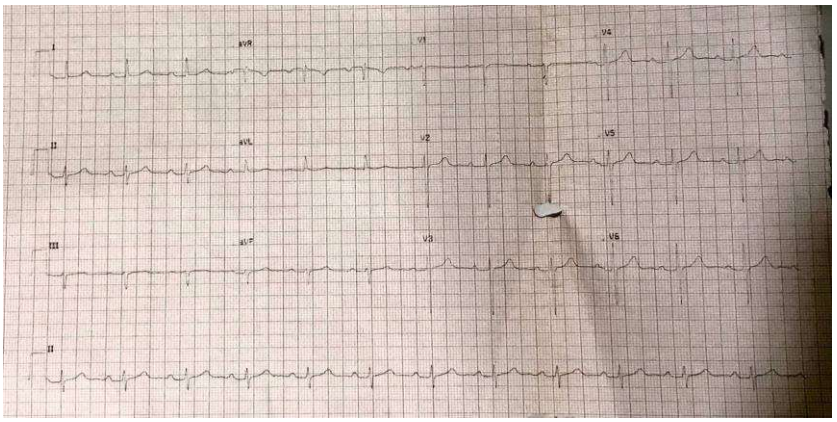

Figure 1: ECG showing prolonged PR interval (first degree AV block) and left axis deviation

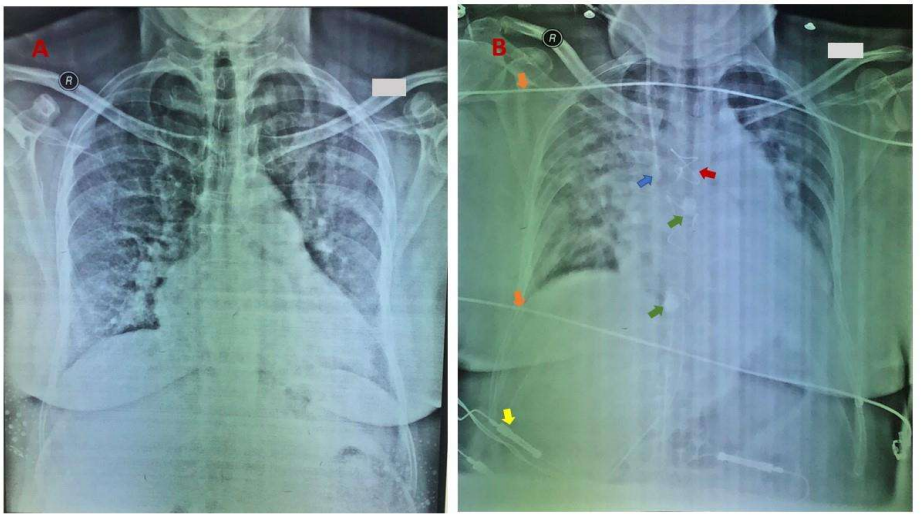

Figure 2: chest x- ray posteroanterior view showing pulmonary congestion and increased cardiothoracic ratio (both A and B); preoperative x- ray (A); post- operative x- ray showing steel- wire for closure of median sternotomy (red arrow), chest drains (green arrows), central venous line (blue arrow), pacemaker wire (yellow arrow), and ECG lead wires (orange arrow) (B).

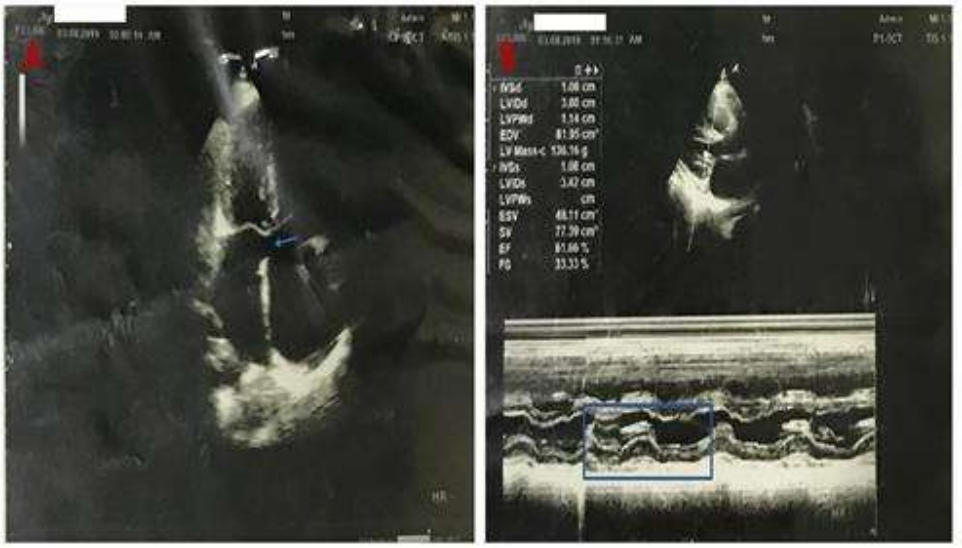

Figure 3: Apical four chamber view showing large ASD primum (line arrow). (A) parasternal long axis view with M-mode showing abnormal movement of mitral valve leaflets and inadequate coaptation (selected area in B).

Table 1: High random blood sugar and ALP; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferse; TSB, total serum bilirubin

Indices	Value
Random blood sugar	233 mg/dl
AST, ALT, ALP	36, 33, 372 U/L respectively
TSB	0.8 mg/dl
Blood urea and serum creatinine	32 and 0.5 mg/dl respectively
Viral screen	Negative

Preoperative and surgical interventions

In the preoperative period, all necessary biochemical and serological investigations were done; findings are shown in Table (1). ECG and Echocardiography were repeated and showed the same results as before.

Intraoperative notes

A surgery was done under general anesthesia with ECG and blood pressure monitoring all on set. The chest wall was opened in layers via median sternotomy then the pericardium was opened through longitudinal pericardiotomy, followed by classical cannulation with single aortic cannula and two separate venous cannulae in the superior and inferior vena cava (SVC and IVC) after administration of intravenous heparin. Cardioplegia cannula was inserted in the aortic root. A total cardiopulmonary bypass was initiated by cross clamping the aorta and arresting the heart via infusion of cardioplegia (Del Nido). The patient was cooled down to 30°C and the heart was immersed in iced water.

The heart was accessed via right atriotomy; then, venting the pulmonary venous return was done with a small vent. An assessment and evaluation of morphology of the defect was done and a primum ASD with cleft in the mitral valve and regurgitation in both AV valves was noticed. The coronary sinus was found intact with no evidence of left superior vena cava (LSVC). The cleft in the mitral valve was repaired by directly interrupted simple suturing using proline 5 zero. The ASD was closed completely by using a non- tanned autologous pericardial patch which was sutured using proline 4 zero. The tricuspid valve was repaired via the suture bicuspidization of the inferior and septal cusps with proline 5 zero. An atrium was closed in two layers and the aortic

cross clamp was removed after careful de-airing. The heart started beating spontaneously; the cardiorespiratory bypass was stopped, and the heparin effect was reversed with protamine sulfate. By then, de-cannulation and thorough hemostasis were achieved and two mediastinal drains were inserted before closing the sternum with steel wires and the rest of the chest wall in layers. The patient was then transferred to the intensive care unit (ICU) for following up and close-monitoring.

In the ICU, the progression was uneventful and the extubation was done on the same day. The cardiac support started in the theatre and continued throughout the ICU period with gradual declining till total weaning on the third postoperative day. Postoperative echocardiography is shown in Figure (4).

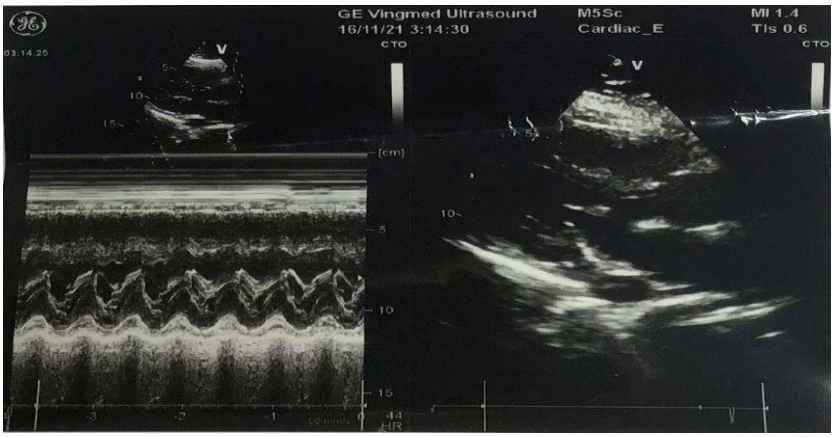

Figure 4: Postoperative echocardiography with parasternal long axis view and M-mode *showing normal movement of mitral valve leaflets and proper coaptation.*

Discussion

This case, like many others which represent a late and undergo surgical correction with immediate good response and marked improvement of patients' conditions in the early postoperative period, gave rise to some loud questions: what happens later? Is this a natural history to go undetected until adulthood? Can surgery fix the problem at any time?

Review of literature related to repair of pAVSD in pediatric patients shows no significant difference in outcome by child age, regarding postoperative mortality and the need for reoperation. The major concern in very young children (1-2 years) is the need for blood transfusion during surgery in those under 15 kg, in addition to the postoperative feeding difficulty that can prolong postoperative hospitalization. Meanwhile, in elder children, the drawback of delay is the risk of developing an AV annular dilation along with left atrial and ventricular dilation that can make repair more difficult. So, from a benefit risk perspective the recommendation was to repair pAVSD between 1-2 years of age [8].

A lot of work has been done to study repair of pAVSD in adults and mostly agree on good outcomes of surgery with no significant surgical mortality as in the present case. However, many problems could accompany late diagnosis and repair, including arrythmia due to heart chamber dilation and remodeling, and increasing the need for left AV valve annuloplasty and replacement with associated increased risk of complete heart block. The need for reoperation in adults is a big concern with 76% and 21% of patients needed reoperation for left AV valve regurgitation and left ventricular outflow tract obstruction, respectively, between 5-10 years postoperatively compared to only 5% and 6% for the same aforementioned reasons in the pediatric study [7,8.9]. So early repair in childhood is always the preferred option.

Out of the data above, one can see a great necessity to find a method to identify these individual cases early in life and prevent delay consequences. Regarding screening for congenital heart disease, a screening program with pulse oximetry for detection of hypoxemia before neonatal discharge is now considered routine for detecting critical congenital heart diseases [10]. Furthermore, prenatal and early postnatal echocardiographic screening is used in groups with a high risk of developing congenital heart disease like those with Down syndrome and other high-risk syndromes and those with abnormal in utero ultrasound findings[11]. Some data discuss the utility of echocardiographic screening for the general population considering it as part of clinical neonatal examination before discharge. However, a high false positive was seen in this very early age which may lead to unnecessary intervention in addition to problems regarding the cost of applying such a screening program on a wide base [12,13]. Further, a study needs to be done to assess the significance of echocardiographic screening after 6 months of age to detect asymptomatic lesions that need early repair.

Conclusion

The undiagnosed congenital heart disease is an important differential diagnosis to keep in mind in adults who present with symptoms of heart failure with no current signs or previous history of ischemic heart disease. More work is needed to establish a well-organized screening program to diagnose these cases early in life and to prevent complications associated with delayed diagnosis.

Conflict of interest

We declare that one of the authors, Dr. Wadhah Mahbuba, is a member of the editorial board in Kufa Medical Journal.

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